



Evaluation of Antioxidant Activity of Branch Extract from *Pouteria campechiana* (Sawo Walanda)

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Abstract. The objective of this research was to assess the antioxidant activity of branch extract (*Pouteria campechiana*) through analysis of bioactive compound content and antioxidant activity. The sample was extracted through maceration, utilizing methanol as the solvent which produced a yield of 6.03%. The quantity of phenolic constituents in the extract was determined using the Folin-Ciocalteu method, with gallic acid as the standard, yielding a result of 195.22 mg GAE/g extract. The quantity of flavonoid constituents was determined using gallic acid as a standard resulting in a value of 830.5 mg QE/g extract. Antioxidant activity was tested using two methods, namely DPPH and ABTS. The results of the DPPH test showed an inhibition percentage of 91% with an IC_{50} value of 3,322 ppm, while the ABTS test showed an inhibition of 99% with an IC_{50} of 2,206 ppm at a concentration of 10,000 ppm. Based on these results, the *Pouteria campechiana* branch extract has very weak antioxidant activity, suggests a limited potential as natural bioactive compound source.

Keywords: antioxidant activity, maceration, *Pouteria campechiana*

Abstrak. Penelitian ini mengkaji kemampuan antioksidan ekstrak batang sawo walanda (*Pouteria campechiana*) melalui analisis senyawa bioaktif yang terkandung di dalamnya serta uji aktivitas antioksidan. Ekstraksi dilakukan menggunakan metode maserasi dengan pelarut metanol yang menghasilkan rendemen sebesar 6,03%. Metode Folin-Ciocalteu digunakan untuk menentukan kadar total fenol pada ekstrak, dengan hasil sebesar 195,22 mg GAE per gram, berdasarkan standar asam galat. Kandungan flavonoid total ditentukan menggunakan asam galat sebagai standar sehingga diperoleh nilai sebesar 830,5 mg QE/g ekstrak. Aktivitas antioksidan diuji menggunakan dua metode, yaitu DPPH dan ABTS. Hasil uji DPPH menunjukkan persentase inhibisi sebesar 91% dengan nilai IC_{50} sebesar 3322 ppm sedangkan uji ABTS menunjukkan inhibisi sebesar 99% dengan IC_{50} 2206 ppm pada konsentrasi 10.000 ppm. Berdasarkan hasil tersebut, batang sawo walanda yang diekstrak terbukti memiliki aktivitas antioksidan yang rendah. sehingga hanya memiliki potensi terbatas sebagai sumber senyawa bioaktif alami.

Kata kunci: aktivitas antioksidan, maserasi, *Pouteria campechiana*

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INTRODUCTION

Indonesia's abundant natural resources have been utilized for generations to support daily needs, including in the field of medicine. Medicinal plants are highly diverse, and their

use continues to increase compared to synthetic drugs (Sahrianti *et al.*, 2025). Around 9 out of 10 Indonesians aged over 15 use traditional medicine in the form of herbal remedies (48%), either as concoctions or through traditional health services (Sahrianti *et al.*, 2025). This indicates that the majority of the

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population still heavily relies on traditional medicine. One type of plant with potential use in traditional treatments is the *sawo walanda*, which has been proven to be used as a remedy for various diseases (Fasna *et al.*, 2019).

Sawo walanda, also known as eggfruit, alkesah, or canistel, and scientifically referred to as *Pouteria campechiana*, is a tropical fruit native to Central America, particularly Mexico. However, it is now widely found and cultivated in various tropical countries, including Indonesia (Fitriansyah *et al.*, 2023). According to a study conducted by Fitriansyah (2023), *sawo walanda* contains secondary metabolites such as phenols, flavonoids, flavonoid glycosides, and terpenoids. The methanol extract of the bark of *sawo walanda* contains caffeine compounds, which belong to the alkaloid group (Rudiana *et al.*, 2021). Various types of secondary metabolites found in *sawo walanda* fruit include alkaloids, glycosides, tannins, terpenoids, and steroids (Mehraj *et al.*, 2015). The highest phenolic and flavonoid content is found in the leaves (Hidayah *et al.*, 2020). These secondary metabolite compounds possess significant pharmacological activities, including antioxidant properties.

Antioxidants are compounds that act as reducing agents and are capable of halting the rate of oxidation reactions. Their mechanism of action includes binding to and preventing the formation of free radicals, as well as inhibiting cellular damage (Rudiana *et al.*, 2021). Free radicals are atoms or molecules that have unpaired electrons in their outer orbitals. They are highly reactive because they can trigger chain reactions by taking electrons from surrounding molecules to stabilize themselves (Muliawati *et al.*, 2016). Continuous and

excessive production of free radicals in the body can lead to lipid oxidation, inactivation of various enzymes, and DNA damage, which may ultimately trigger cellular changes and become the initial cause of diseases such as cancer (Rudiana *et al.*, 2021). Antioxidants are compounds capable of donating one or more electrons to free radicals, thereby neutralizing their activity (Muliawati *et al.*, 2016). The development of plant-based antioxidant sources continues to be pursued as a preventive measure against the emergence of various diseases (Rudiana *et al.*, 2021). Various studies have shown that parts of the *sawo walanda* plant can serve as natural antioxidants.

The antioxidant activity of *sawo walanda* fruit pulp using the DPPH method shows an IC_{50} value of 2656 ppm (Muliawati *et al.*, 2016). *Sawo walanda* fruit extract can be utilized in the tofu-making process as a natural antioxidant source, thereby enhancing the nutritional value and health potential of the tofu (Wibowo *et al.*, 2021). *Sawo walanda* leaf extract has shown significant potential as a natural antioxidant (Fitriansyah *et al.*, 2023). The antioxidant activity of *sawo walanda* extracts from the peel, pulp, seeds, and leaves has demonstrated strong activity, with a DPPH IC_{50} value of 50 $\mu\text{g/mL}$ (Hidayah *et al.*, 2020). Based on these findings, each part of the *sawo walanda* plant exhibits different levels of antioxidant activity. However, the antioxidant activity of the *sawo walanda* branch has not yet been reported.

In this study, the extraction of *sawo walanda* branch was carried out using the maceration method with methanol as the solvent. The phenolic content of the extract was then measured using the Pourmorad method, and the flavonoid content was measured using

the Chang method. The antioxidant activity of the *sawo walanda* branch extract was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and the ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) method. Hence, the objective of this study is to assess the antioxidant activity of *Pouteria campechiana* branches with DPPH and ABTS assays.

MATERIAL AND METHODS

Materials

The materials used in this study included *sawo walanda*'s branch obtained from the Mojoroto area, Kediri City, East Java, Indonesia; methanol from Merck; distilled water; Folin-Ciocalteu reagent from Sigma Aldrich; Na_2CO_3 from Merck; AlCl_3 from Merck; DPPH from Sigma Aldrich; ABTS from Merck; gallic acid from Merck; and $\text{K}_2\text{S}_2\text{O}_8$ from Merck—all obtained from the Microorganism Chemistry Laboratory, Department of Chemistry, ITS.

Instrumentation

The equipment used in this study included a blender from Philips, a Retsch 200 mesh test sieve, a 100 mL Erlenmeyer flask from Iwaki, a 100 mL measuring cylinder from Duran, filter paper from Whatman, a rotary evaporator from Buchi, micropipettes from Socorex, an incubator, and a UV-Vis spectrophotometer (Shimadzu UV-1280).

Procedure

Sample preparation and extraction

The *sawo walanda* branch were washed with clean running water, coarsely chopped, and then dried. The dried coarse sample was ground into a fine powder, and 15 grams of the sample were taken for extraction using the maceration method, performed three times over a period of twenty-four hours in methanol. The resulting macerate was concentrated using a rotary evaporator to obtain a thick methanolic extract. The extraction process illustrated in Figure 1.



Figure 1. The extraction process of sawo walanda's branch

Total phenolic content (TPC)

The phenolic content was analyzed using the Folin-Ciocalteu method (Noreen et al.,

2017). A volume of 0.1 mL of 1000 ppm methanolic extract was taken and mixed with 0.5 mL of 10% Folin-Ciocalteu reagent. The

mixture was incubated at room temperature for 5 minutes. After incubation, 0.4 mL of 7.5% Na₂CO₃ was added, and the mixture was incubated at 40°C for one hour. The absorbance of the test solution was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. Gallic acid was used as the standard or positive control for phenol.

Total flavonoid content (TFC)

The flavonoid content was analyzed using the Chang method (Chang et al., 2002). A volume of 0.5 mL of 1000 ppm methanolic extract was mixed with 0.5 mL of 2% AlCl₃ solution in methanol. The test solution was incubated at room temperature for one hour. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 415 nm. In this study, gallic acid was used as the standard or positive control for flavonoids.

Antioxidant assay using the DPPH method

A stock solution of 10,000 ppm was prepared from the concentrated extract. The test solution was prepared by adding 33 µL of the sample to 1 mL of 0.006% DPPH solution in methanol. The mixture was homogenized and incubated at 37°C for 20 minutes. The absorbance of the sample was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The test was performed in triplicate. The percentage of inhibition was calculated using Equation (1).

$$\% \text{inhibition} = \frac{A_b - A_x}{A_b} \times 100\% \dots (1)$$

where,

A_b = Blank absorbance

A_x = Sampel absorbance

If the % inhibition of the concentrated extract exceeds 50%, the IC₅₀ test is conducted. A 10,000ppm stock solution is serially diluted to obtain concentrations of 5000,

2500, 1250, 625, 312.5, 156.25, 78.125, and 39.063 ppm. For each concentration, 33 µL is pipetted and mixed with 1 mL of 0.006% DPPH solution in methanol. The test solutions are thoroughly mixed and incubated at 37°C for 20 minutes. Absorbance is measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The analysis is performed in triplicate to ensure the reliability of the results, and the % inhibition is calculated using Equation (1). Gallic acid is used as the positive control.

Antioxidant assay using the ABTS method

The ABTS solution was prepared by dissolving 19.2 mg of ABTS in 5 mL of distilled water. Separately, 3.33 mg of K₂S₂O₈ was dissolved in 88 µL of distilled water. The two solutions were mixed and stored in a dark room for 12–16 hours, then diluted with methanol to obtain a solution with an absorbance of 0.7 (±0.2), which was used as the ABTS working solution. A stock solution of 10,000 ppm was prepared from the concentrated extract. A volume of 10 µL of the sample solution was added to 1 mL of the ABTS solution, and the mixture was homogenized and incubated at 30°C for 4 minutes. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 734 nm. The test was performed in triplicate. The percentage of inhibition was calculated using Equation (1).

If the % inhibition of the concentrated extract exceeds 50%, an IC₅₀ test is conducted. A 10,000ppm stock solution is serially diluted to obtain concentrations of 5000, 2500, 1250, 625, 312.5, 156.25, 78.125, and 39.063 ppm. For each concentration, 10 µL is pipetted and mixed with 1 mL of the ABTS solution. The test solution is homogenized and incubated at 30°C for 4 minutes. Absorbance is measured using a

UV-Vis spectrophotometer at a wavelength of 734 nm. The test is performed in triplicate. The percentage of inhibition is calculated using Equation (1).

RESULT AND DISCUSSION

Sampel Extract

A total of 15 grams of powdered *sawo walanda* branches were used for extraction using the maceration method with methanol, resulting in 0.940 grams of thick extract (yield of 6.03%). In a study by Rudiana (2021), the methanolic extract of *sawo walanda* bark showed the highest yield at 10.45%, compared to 0.77% with n-hexane and 0.83% with ethyl acetate. The high yield of the methanolic extract indicates that it contains a large number of polar compounds, such as flavonoids and polyphenols (Rudiana *et al.*, 2021).

The basic structure of flavonoids consists of two aromatic rings connected by a pyran ring, making them more polar. Therefore, when a polar solvent such as methanol is used for extraction, the resulting yield tends to be higher than when using non-polar solvents (Doloking *et al.*, 2022). This is in accordance with the principle of "like dissolves like," where polar compounds tend to dissolve in polar solvents, and the molecular interactions that occur include hydrogen bonding or dipole–dipole interactions (Zhuang *et al.*, 2021).

Total phenol content

The results of the phenolic content test showed that the *sawo walanda* branch extract contained 195.22 mg GAE/g of phenolic compounds. The linear regression of gallic acid is shown in the graph in Figure 2. This linear regression was used to calculate the total phenolic content of the *sawo walanda* branch extract, as presented in Table 1.

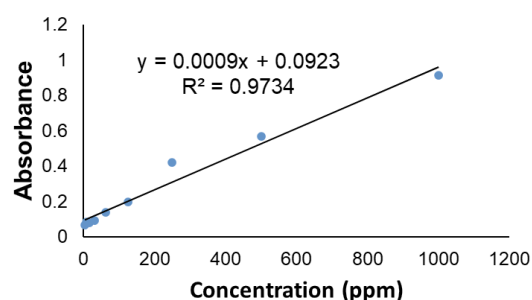


Figure 2. Standard curve of TPC (gallic acid)

Table 1. Total phenolic content (TPC)

Repetition	Absorbance	TPC Content (ppm)	TPC Content (mg GAE/g)
1	0,254	179,666	179,666
2	0,28	208,555	208,555
3	0,27	197,444	197,444
Average	0,268	195,222	195,222

This is consistent with the study by Hidayah (2019), which found that the peel, fruit, seeds, and leaves of *sawo walanda* contain phenolic compounds, suggesting that other parts of the plant, such as the branches, also contain phenolics, with a level of 195.22 mg GAE/g. In a study by Fitriansyah (2023), the total phenolic content of *sawo walanda* leaf extract, calculated using the gallic acid standard curve regression, was 127 mg GAE/g. The phenolic content of *sawo walanda* fruit extract was reported to be 192.6 mg GAE/g (Wibowo *et al.*, 2021).

Phenolic compounds are the most commonly found compounds in plants and are responsible for various biological activities. These activities include defense responses such as anti-aging, anti-inflammatory, and antioxidant effects (Lin *et al.*, 2016).

Total flavonoid content

The results of the flavonoid content test showed that the *sawo walanda* branch extract

contained 830 mg QE/g of flavonoid compounds. The linear regression of quercetin can be seen in the graph in Figure 3. This linear regression was used to calculate the total flavonoid content of the *sawo walanda* branch extract, as presented in Table 2.

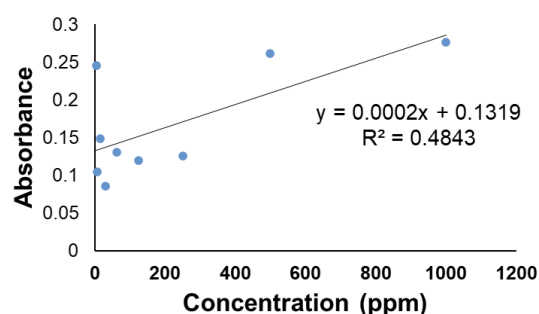


Figure 3. Standard curve of TFC (gallic acid)

Table 2. Total flavonoid content (TFC)

Repetition	Absorbance	TFC Content (ppm)	TFC Content (mg QE/g)
1	0.254	179.666	179.666
2	0.280	208.555	208.555
3	0.270	197.444	197.444
Average	0.298	830.5	830.5

The linear regression obtained showed an R^2 value of only 0.4843. A lower R^2 value indicates a higher error in the data (Kartiningrum *et al.*, 2022). This is likely due to the use of an inappropriate standard in the preparation of the TFC calibration curve; quercetin should have been used as the control. Standard curves for flavonoid testing typically use quercetin (Wibowo *et al.*, 2021). As a flavonoid compound with strong biological activity, quercetin is chosen as a reference standard due to its ability to neutralize free radicals (Fitriansyah *et al.*, 2023). However, in this study, gallic acid was used as the calibration standard due to material limitations.

The total flavonoid content in the *sawo walanda* branch extract was 830.5 mg QE/g. Although the results may not be entirely accurate, they still indicate the presence of flavonoid compounds in the sample. This is consistent with the findings of Hidayah (2019), who reported that the peel, fruit, seeds, and leaves of *sawo walanda* contain flavonoid compounds. *Sawo walanda* contains groups of phenols, flavonoids, flavonoid glycosides, and terpenoids (Fitriansyah *et al.*, 2023). The highest phenolic and flavonoid content is found in the leaves (Hidayah *et al.*, 2020). The flavonoid content in *sawo walanda*'s branch extract is 0.37 g QE/100 g (Fitriansyah *et al.*, 2023). While in the leaf extract it is 0.8 g QE/100 g (Hidayah *et al.*, 2020).

Antioxidant Assay Using the DPPH Method

The antioxidant activity test of *sawo walanda* extract using the DPPH method at the highest concentration of 10,000 ppm showed an inhibition of 91%, as presented in Table 3. This result is in line with the study by Uuh-Narvaez (2023), which reported a DPPH inhibition of 92.15% for *P. campechiana* fruit. The IC_{50} of the *sawo walanda*'s branch extract was further calculated using linear regression from the serial dilution concentrations, as shown in the graph in Figure 4.

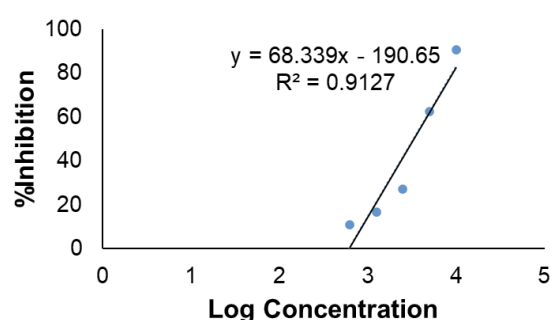
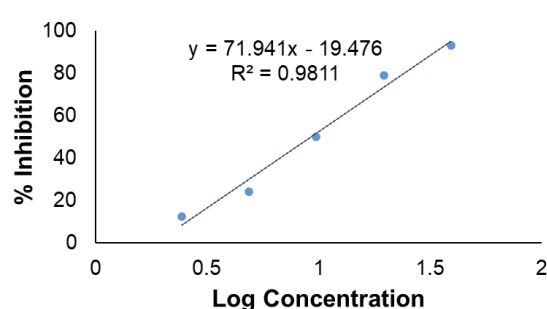


Figure 4. DPPH graph of sample extract

Table 3. Percentage inhibition of sample extract using the DPPH method

Repetition	Blank	Sample	Blank - sample	Inhibition (%)
1	0,666	0.056	0.610	92
2	0,662	0.077	0.585	88
3	0,672	0.051	0.621	92
Average				91

In this experiment, gallic acid was used as a positive control. The results of the antioxidant activity test of gallic acid using the DPPH method are shown in Figure 5.

**Figure 5.** DPPH graph of gallic acid**Table 4.** Comparison of IC₅₀ values between sample extract and gallic acid

Extract	IC ₅₀ (ppm)
<i>Sawo walanda</i> 's branch	3322
Gallic acid	9.24

The IC₅₀ value of *sawo walanda* branch extract, calculated using linear regression from the graph in Figure 3, was found to be 3322 ppm. The IC₅₀ value of *sawo walanda* fruit pulp was reported to be 2656 ppm, which indicates very weak antioxidant activity (Muliawati *et al.*, 2016). In the study by Fitriansyah (2023), the IC₅₀ of *sawo walanda* leaf extract using the DPPH method was 1.18 µg/mL, while the fruit extract was 3.55 µg/mL. The IC₅₀ of gallic acid, used as a positive control and calculated using the linear regression from Figure 4, was found to be 9.24 ppm. This is consistent with the study by Gultom (2021), which reported the IC₅₀ of gallic acid to be 8.93 µg/mL. A comparison of

the IC₅₀ values of *sawo walanda* branch extract and gallic acid using the DPPH method is presented in Table 4.

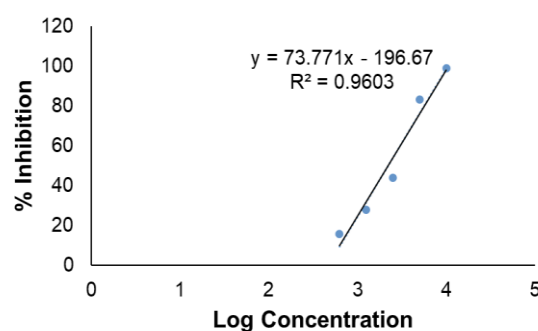
Compounds such as flavonoids, phenolics, and stilbenoids, which contain hydroxyl groups (-OH) in their structures, act as natural antioxidants due to their ability to scavenge free radicals (Fernandez-Panchon *et al.*, 2008). The presence of hydroxyl groups (-OH) in a molecule enables the compound to function as a free radical inhibitor (Fitriansyah *et al.*, 2023). The *sawo walanda* branch extract exhibits antioxidant activity because it contains phenolic and flavonoid compounds, which were also tested in this study.

Antioxidant assay using the ABTS method

The antioxidant activity test of *sawo walanda* branch extract using the ABTS method at the highest concentration of 10,000 ppm showed an inhibition of 99%, as presented in Table 5. The IC₅₀ of the *sawo walanda* branch extract was further calculated using linear regression from the serial dilution concentrations (Figure 6).

Table 5. Percentage inhibition of sample extract using the ABTS method

Repetition	Blank	Sample	Blank - sample	Inhibition (%)
1	0.635	0.004	0.631	99
2	0.651	0.006	0.645	99
3	0.654	0.008	0.646	99
Average				99

**Figure 6.** ABTS graph of the sample

In this experiment, gallic acid was used as the positive control. The results of the antioxidant activity test of gallic acid using the ABTS method are shown in Figure 7.

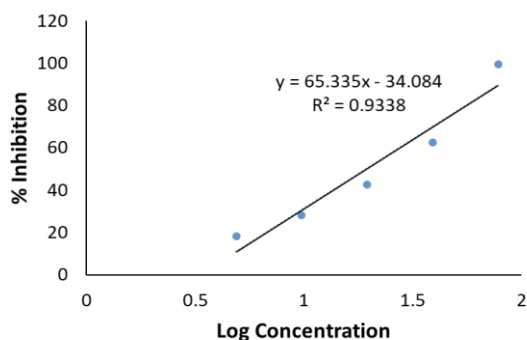


Figure 7. ABTS graph of gallic acid

The IC_{50} value of *sawo walanda* branch extract, calculated using linear regression from the graph in Figure 5, was found to be 2206 ppm. The IC_{50} value of ABTS extract from *P. campechiana* branches was reported as 9 $\mu\text{g/mL}$ (Do et al., 2023). The IC_{50} of gallic acid, used as a positive control and calculated using linear regression from the graph in Figure 6, was 19.36 ppm. The IC_{50} of ABTS for gallic acid was reported as 1.03 $\mu\text{g/mL}$ (Lee et al., 2015). A comparison of the IC_{50} values of *sawo walanda* branch extract and gallic acid using the ABTS method is presented in Table 6.

Tabel 6. Comparison of IC_{50} values between sample extract and gallic acid using the ABTS method

Extract	IC_{50} (ppm)
<i>Sawo walanda's</i> branch	2206
Gallic acid	19.36

The hydroxyl groups and aromatic rings in phenolics and flavonoids play a crucial role in antioxidant activity. Hydroxyl groups can donate hydrogen atoms (H) to neutralize free radicals by converting reactive radicals into more stable molecules. This hydrogen atom transfer mechanism is central to antioxidant

function. The ability of free hydroxyl groups to donate hydrogen greatly influences a molecule's capacity to scavenge free radicals (Charlton et al., 2023). When hydroxyl groups donate hydrogen to free radicals, the resulting antioxidant radical form is stabilized by resonance, especially in aromatic structures such as phenolics and flavonoids. This stabilization prevents further chain radical reactions, thereby enhancing antioxidant efficiency (Yan et al., 2024).

CONCLUSION

The *sawo walanda* branch extract obtained through maceration using methanol as the solvent shows very weak antioxidant activity, suggests a limited potential as natural bioactive compound source. The extract yield of 6.03% contains bioactive compounds with a total phenolic content of 195.22 mg GAE/g and total flavonoid content of 830.5 mg QE/g. Antioxidant activity tests using the DPPH and ABTS methods demonstrated high inhibition rates of 91% and 99%, respectively, at a concentration of 10,000 ppm, with IC_{50} values of 3322 ppm (DPPH) and 2206 ppm (ABTS). To improve the accuracy of total flavonoid content measurement, it is recommended to use quercetin as the reference standard instead of gallic acid. This is because quercetin better represents the chemical structure of flavonoids, providing more specific and relevant results for the compounds analyzed.

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