Evaluation of *Peronema canescens* Leaves Extract: Fourier Transform Infrared Analysis, Total Phenolic and Flavonoid Content, Antioxidant Capacity, and Radical Scavenger Activity

Tonny Maigoda1*, Judiono Judiono2*, Dicki Bakhtiar Purkon3, Ayu Nala El Muna Haerussana4, Gurid Pramintarto Eko Mulyo2

1Department of Nutrition, Poltekkes Kemenkes Bengkulu, Bengkulu, Indonesia; 2Department of Nutrition, Poltekkes Kemenkes Bandung, Bandung, Indonesia; 3Department of Pharmacy, Poltekkes Kemenkes Bandung, Bandung, Indonesia

Abstract

**BACKGROUND:** *Peronema canescens* (Sungkai) leaves have been popular in Indonesia which contain various bioactive compounds with empirical therapeutic efficacy in dealing with COVID-19 and various other diseases. Total phenolic and flavonoid content and antioxidant activity using the DPPH method from *P. canescens* leaf extract have not been studied much.

**AIM:** This research has several objectives. The first is to compare the results of qualitative phytochemical analysis of the ethanol extract of the leaves of *P. canescens* (EEPL). The second is to measure the total phenol and flavonoid content. The third is to test the FTIR and antioxidant activity of the ethanol extract of *P. canescens* leaves in vitro using the DPPH method.

**METHODS:** Fresh plant material and simplicia, ethanol extract extracted by maceration method using 96% ethanol as solvent from *P. canescens*. The Dragendorff’s and Mayer test carried out the qualitative phytochemical analysis, FeCl3 test, Salkowski method, Liebermann–Burchard method, foam test, and NaOH reagent. The total phenolic and flavonoid levels were tested using the Folin–Ciocalteu method. In vitro antioxidant activity was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method.

**RESULTS:** The results of qualitative phytochemical screening showed that alkaloids, flavonoids, saponins, tannins, and steroids were detected in the extract of *P. canescens*. The spectra from the FTIR test results showed various absorbance peak values indicating the bonding of specific functional groups, namely: 418.12, 599.94, 666.67, 1036.39, 1159.52, 1224.16, 1348.95, 1454.19, 1600.87, 1732.00, 2923.13, and 3353.01 cm⁻¹. In the test results, total phenolic content was as much as 116.7865 ppm. The spectra from the FTIR analysis showed various absorbance peak values indicating the bonding of specific functional groups, namely: 418.12, 599.94, 666.67, 1036.39, 1159.52, 1224.16, 1348.95, 1454.19, 1600.87, 1732.00, 2923.13, and 3353.01 cm⁻¹. In the test results, total phenolic content was as much as 5.64% (mgEAG/g) and total flavonoid content of 142,247 mgEQ/g in a sample of 1 mg extract, which was equivalent to 1 mg quercetin. EEPL has antioxidant activity with the DPPH IC50 method of 116.7865 ppm.

**CONCLUSION:** The data obtained at this time can contribute to the exploitation of *P. canescens* leaves in the future as one of the nutraceutical products, supplements, and herbal medicines by specific industries related to improving the health status of the world community. The higher the bioactive substance in preparation, the more significant the effect of the pharmacological efficacy response. *P. canescens* ethanol extract has good total phenolic content, total flavonoid content, and antioxidant action.

Introduction

Oxidative stress produced by high concentrations of free radicals is vital in inducing several degenerative diseases such as cancer, aging, rheumatoid arthritis, autoimmune, neurodegenerative, cardiovascular disorders, and cataracts [1]. The human body has many mechanisms (pathways) to combat oxidative stress by naturally producing endogenous compounds with antioxidant properties or supplied externally by functional foods (nutraceuticals) or supplements. Therefore, antioxidant compounds can affect biological responses in dealing with the causes of free radicals and reduce the risk of degenerative diseases [2]. The side effects of using herbal medicines tend to be fewer than conventional medicines (patent medicines).

However, this must still be scientifically proven and safe for use for humans. Plant constituents with antioxidant activity have been reported to protect biological systems against free radicals (oxidative stress). Antioxidants from herbal ingredients increase antioxidant capacity and reduce the risk of various diseases such as cancer, heart disease, and stroke. Secondary metabolites such as phenolic compounds and flavonoids from multiple parts of plant materials have been reported as strong free radical scavengers in leaves, fruit, seeds, roots, and bark [2], [3]. The use of herbal ingredients as medicinal products and functional food (nutraceuticals) has been known for thousands of years, as evidenced by various sources obtained, such as ancient books of medicine and various notes on the walls of monuments as a heritage in the world’s great societies [4].
Peronema canescens (synonym: Sungkai/Jatibarang in Indonesia) belongs to the Verbenaceae family. One of the places to grow this type of plant is in Bengkulu Province and several regions in Indonesia. This plant is commonly used as an ornamental plant bordering the house. In East Kalimantan, the Dayak tribe (one of the tribes in Indonesia) uses the leaves of P. canescens as traditional medicine (Jamu) to treat fever, influenza virus infection, stomachache, and antibacterial around the mouth and skin. In South Sumatra and Lampung Province, young leaves of P. canescens are used as antiplasmodial, anti-fever, and anti-inflammatory [5], [6], [7]. P. canescens leaves are herbal ingredients with many secondary metabolite components such as phenolic compounds, flavonoid compounds, and terpenoids, which have many benefits for human health [5]. The study shows that information regarding the antioxidant activity of the ethanolic extract of the leaves of P. canescens is still limited. One of the antioxidant testing methods used is the DPPH (2,2-diphenylpicrylhydrazyl) free radical scavenging method [8]. The DPPH method is relatively easy and inexpensive, so it is more commonly used.

The purpose of this study was to carry out a qualitative phytochemical screening to assess the total phenolic and flavonoid content, to test the FTIR analysis and antioxidant ability of the ethanol extract of P. canescens leaves using the DPPH (free radical scavenging) method. The results provide valuable information for future studies on P. canescens leaves for commercial purpose to improve public health status in nutraceutical dosage forms, supplements, or herbal medicine.

Materials and Methods

Plant materials

The sample used in this study was the ethanol extract of the leaves of P. canescens (EEPL) leaves in a dry extract with constant weight. The fresh ingredients for adult P. canescens leaves (synonym name: Sungkai in Indonesia) were obtained in Bengkulu City, Bengkulu Province, Sumatra Island, Indonesia. About 96% ethanol solvent obtained from PT. Brataco Chemical, Bandung. Meanwhile, the quercetin compound was obtained from Merck® (Darmstadt, Germany). The pro-analytical quality level of all chemicals is used in this study.

Preparation of ethanolic extract

The extraction process was made using the maceration method of 96% ethanol as solvent. P. canescens leaves as much as 500 g were washed thoroughly, and then, the leaf size was reduced (chopped) to form small pieces of leaves and put into 5 L of ethanol [9]. The mixture was stirred several times for 3 days. Every day, the filtrate is filtered, and a new solvent is replaced after 24 h. Next, after the filtration process, the filtrate was dried in a rotary vacuum evaporator (temperature at 50°C, rotation at 80/min) and evaporated to obtain a dry/thick extract with constant weight using a water bath. The dry filtrate is stored in a tightly closed vial container and stored in the refrigerator until further use.

Alkaloid test

Mayer’s test

The sample (100 mg) was dissolved in 10 mL of solvent, then filtered to form a filtration. Filtration (2 mL) was added with HCl (acid) and a few drops of Mayer’s reagent/reagent. The formation of a white or brownish-yellow precipitate indicates the presence of alkaloids [10].

Wagner’s test

A sample of 100 mg is dissolved in 10 mL of water solvent and then mixed with 8–10 drops of Wagner’s reagent. The formation of a brick red or brown precipitate indicates a positive result [11].

Flavonoid tests

Shinoda test: A total of 2–3 mL of liquid extract filtrate is added with Mg metal (magnesium). The 2 N HCl concentrate and amyl alcohol were added, forming a color change. The appearance of a brownish-yellow or magenta color indicates the presence of flavonoids [10].

Saponin tests

Foam test/froth method: The dry extract in a test tube is dissolved in 10–20 mL of distilled water until the sample is submerged and heated/boiled for 2–3 min. The solution was then cooled and shaken. The appearance of foam for ± 10–15 min indicates the presence of saponin compounds [10].

Tannin and polyphenolic compound test

1% FeCl₃ test

A sample of 100 mg is dissolved in 10 mL of solvent, then filtered. The filtrate (2 mL) was added with 1 mL of 1% FeCl₃. The formation of a green to slightly blackish precipitate indicates the presence of tannins and polyphenols [10].
1% gelatin test

0.1 g of dry extract was added with 10 mL of distilled water, then heated (boiled) for several minutes. Then, the filter results (filtrate) were added with 2 mL of 1% gelatin solution containing NaCl. If a white precipitate is formed, it indicates the presence of tannins and phenolic components [10].

Steroid test

Liebermann–Burchard test: 50 mg extract sample was extracted with chloroform and filtered. The result of filtering (filtrate) as much as 2 mL formed then added 1–2 mL of acetic anhydride and two drops of concentrated H2SO4 from the side of the test tube. The color formed is red, blue, and green, indicating sterol compounds [10].

Procedure for FTIR analysis

1. Fourier-transform infrared (FTIR) spectroscopy spectra were recorded in the wavelength range of 4000–400 cm⁻¹ using potassium bromide (KBr) on a Thermo Fisher Scientific® FTIR spectrometer. The prepared KBr pellets were used for this study.
2. To obtain a homogeneous powder sample for FTIR analysis, samples of dry extract of P. canescens leaves were prepared by grinding.
3. A total of 1 mg of dry extract powder and 500 mg of KBr were weighed. The dry extract powder was encapsulated in KBr pellets to prepare translucent sample discs. Then, the KBr pellets were scanned between the range of 4000 and 400 cm⁻¹.
4. FTIR spectroscopy as a non-destructive technique has provided a powerful tool for identifying functional groups present in compounds by interpreting annotated absorption spectra.

Total phenolic compound analysis

Determination of total phenolic content examination using the Folin–Ciocalteu test method as described by Erkekoglou et al. (2017) and Salem et al. (2013) [3], [12] used a 15 mg/10 mL P. canescens leaf extract solution. The test sample was added 1 mL of Folin–Ciocalteu phenol reagent in the measuring flask, then shaken. After 5 min, 10 mL of the 70 g/L Na2CO3 solutions was added to the mixture. The solution was diluted to 25 mL with the addition of distilled H2O and mixed. After incubation for 90 min at room temperature, the absorbance of the prepared reagent blank was determined at 750 nm using a UV spectrophotometer. The results are expressed as a percentage (%) equivalent to gallic acid, GAE (mg GAE/g dry extract or dried leaves).

Total flavonoid compound analysis

Analysis of the total flavonoid content of the ethanolic extract of the leaves of P. canescens is using the aluminum chloride reagent colorimetric method. This method was adapted from Nurcholis et al. (2021) with slight changes. A total of 50 L of the extract were placed on a 96-microwell plate containing 10 L 10% aluminum chloride, 130 L 96% ethanol, and 10 L 1 M sodium acetate 1 M. The mixture was incubated for 40 min at room temperature in a dark room. The absorbance at 415 nm was measured using a UV–visible spectrophotometer. The total flavonoid content was expressed as mg of quercetin equivalent (QE) per gram dry weight (mgEq/g dry weight) through a calibration curve with quercetin. All samples were performed in three replications [1].

Measurement of antioxidant activity

in vitro (DPPH method)

The free radical scavenging activity of the DPPH method was determined by the method described by Sridhar and Charles (2018) with slight modifications [13]. For DPPH assay, 0.70 mL of sample or standard extract (ascorbic acid) with varying concentrations (0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mg/mL) was added to the DPPH methanol solution with the same volume (100 M). The mixture was shaken vigorously and incubated for 20 min (left) in a dark room at room temperature. The decrease in absorbance was measured at 515 nm against a methanol blank without DPPH using a UV–visible spectrophotometer. The measured absorbance for the control solution was in the range of 0.74 ± 0.01. The percentage of inhibition of DPPH color change was calculated using the following equation:

\[
\% \text{Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Acontrol is the control absorbance and Aextract is the extract absorbance.

Results

P. canescens leaf Simplicia is shown in Figure 1. (c) 1.05 kg of fresh leaf material has been obtained as much as 4 kg, which has been washed, drained, chopped, and arranged when drying by air drying for ± 2–3 weeks. The ratio of Simplicia produced from fresh P. canescens leaves is 1:4. The moisture content of P. canescens leaf Simplicia obtained was 8.30 ± 0.12%. The water content test was carried out using the gravimetric method with 3 times weighing replication when determining constant weight.
Five hundred grams of *P. canescens* leaves using 96% ethanol as solvent extraction process. The ratio used is 1:10. The evaporation process of the liquid extract solvent was carried out using a rotary vacuum evaporator which was then thickened with a water bath. The results of the dry extract were blackish-green with a constant weight of 100,784 g so that the percentage (%) of extract yield is 20.16% w/w.

The results of the phytochemical analysis of leaves of *P. canescens*

The qualitative phytochemical screening test results on the ethanol extract of the leaves of *P. canescens* jack, as shown in Table 1, which contains alkaloids, flavonoids, saponins, tannins, and steroids. Screening or screening of phytochemicals from these extracts was carried out by standard methods, as mentioned by the method guidelines from the Ministry of Health of the Republic of Indonesia (2000), Siregar et al. (2021), and WHO (2011) [14], [15], [16]. The results of qualitative phytochemical screening showed the presence of a positive group of compounds which included alkaloids, flavonoids, saponins, tannins and polyphenolic compounds, as well as steroids.

The results of FTIR spectroscopy analysis of *P. canescens* leaves

In the results of the EEPL FTIR spectrum, characteristic absorption peaks observed at wavelength 1600 cm\(^{-1}\) indicates the presence of NH (amide) bonds; at 1036.39, 1159.52, 1224.16, and 1348.95 cm\(^{-1}\) are shown CN (amine) strain; at 1454.19 cm\(^{-1}\), the strain is C=C; at 3353.01 cm\(^{-1}\) shows the NH (secondary amine) strain, aliphatic -NH strain, or OH strain; at 2932 cm\(^{-1}\) to show CH stretch, aliphatic -CH stretch or CH stretch; at 1036.39 cm\(^{-1}\) to indicate -S=O or CO- strain; on 666.67 cm\(^{-1}\) to show C=C (bent); and at 1159.52 and 1224.16 cm\(^{-1}\) to represent CO phenol. The results of the FTIR spectra of the EEPL sample are shown in Table 2.

Total phenolic content of ethanolic extractions of *P. canescens* leaves (EEPL)

The results of the total phenolic content of the dry EEPL are shown in Table 3.

Total flavonoid content of EEPL

Calculation of the concentration of quercetin compounds from the ethanol extract of *P. canescens* leaf extract (ppm):

\[
y = bx + a
\]

Table 1: Results of phytochemical screening of the ethanol extract of *Peronema canescens* leaves

| Compound group | Test method | Result | *Peronema canescens* leaves |
|---------------|-------------|--------|---------------------------|
| Alkaloid       | a. Mayer reagent | A white precipitate is formed | + |
|                | b. Wagner reagent | Formation of color change to brick red | + |
|                | c. Dragendorff’s reagent | No change | − |
| Flavonoid      | Aquadest, Mg powder+HCl | Brownish-yellow | + |
|                | 2N+amyl alcohol | | |
| Saponin        | Aquadest, then shaken+HCl 2N | A stable foam of 1–10 cm is formed for several minutes (despite the addition of 1% HCl) | + |
| Tannin and polyphenolic compound | Aquadest, heated+FeCl\(_3\) 1% | There is a change in color to brownish-green | + |
| Steroid        | Ether/chloroform+Liebermann-Burchard reagent | A white precipitate is formed | + |

+: Positive, −: Negative.
0.618 = 0.0111x + 0.3037
0.0111x = 0.618 – 0.3037
0.0111x = 0.3146
x = 28.4493 ppm

Calculation of total flavonoid content of P. canescens leaf ethanol extract (mg Eq/g):

\[ \text{Absorbance (mg Eq/g)} = c \times v \times fp/g \]

= 142.247 mg EQ/g P. canescens leaf ethanol extract

As a basis for quantitative determination, the total flavonoid content in the ethanolic extract of the leaves of P. canescens (Figure 2 and Table 4) was determined using aluminum chloride in a colorimetric method. The results were derived from the calibration curve (\( y = 0.0111x + 0.3037, R^2 = 0.9952 \)) of quercetin (0–100 g/mL) (Table 4) and expressed in terms of QE per gram by weight of dry extract. The total flavonoid content in the EEPL was 142,247 mg QE/g.

**Discussion**

The fresh sample of P. canescens leaves shown in Figure 1 was the main source of ethanol extract in various in vitro tests in this study. The water content of certain extracts, which proved to be less than 15%, confirms its well-maintained quality as discussed in the US Pharmacopeia (2017). Its extracts can be applied to various nutraceutical products, herbal medicines, and cosmetics [17]. The percentage yield of EELP obtained (20.16% w/w) showed that various bioactive components of P. canescens leaf Simplicia were extracted with 96% ethanol as a universal solvent. Ethanol is a good solvent for extracting polyphenolic compounds and flavonoids and is safe for human consumption [18], [19]. The extraction method carried out on this plant is the maceration method. Maceration is an extraction method to obtain antioxidant compounds from plants [19]. This extraction method also has various advantages – first, good solvent penetration into plant particles, second, low extraction temperature, third, simpler, and fourth, it is more convenient and relatively cheaper instrumentation [20].

**Figure 2: The results of the standard calibration curve for the flavonoid compound quercetin**

| Concentration (ppm) | Replication | Absorbance     | Mean ± Standard Deviation |
|---------------------|-------------|----------------|----------------------------|
| 10                  | 1           | 0.403          | 0.404 ± 0.0038             |
|                     | 2           | 0.406          |                            |
|                     | 3           | 0.407          |                            |
| 20                  | 1           | 0.542          | 0.541 ± 0.0012             |
|                     | 2           | 0.540          |                            |
|                     | 3           | 0.540          |                            |
| 30                  | 1           | 0.621          | 0.620 ± 0.0015             |
|                     | 2           | 0.618          |                            |
|                     | 3           | 0.620          |                            |
| 40                  | 1           | 0.757          | 0.757 ± 0.0015             |
|                     | 2           | 0.759          |                            |
|                     | 3           | 0.756          |                            |
| 50                  | 1           | 0.868          | 0.868 ± 0.0006             |
|                     | 2           | 0.868          |                            |
|                     | 3           | 0.868          |                            |
| 60                  | 1           | 0.954          | 0.954 ± 0.0015             |
|                     | 2           | 0.954          |                            |
|                     | 3           | 0.953          |                            |

**Table 4: Absorbance results from standard calibration dilution of quercetin flavonoid compounds and Peronema canescens leaf extract samples using UV spectrophotometer**

| Concentration (ppm) | Replication | Absorbance     | Mean ± Standard Deviation |
|---------------------|-------------|----------------|----------------------------|
| Peronema canescens leaf sample | 1 | 0.619 | 0.619 ± 0.0012 |
|                     | 2           | 0.619          |                            |
|                     | 3           | 0.617          |                            |

**Figure 3: Fourier transform infrared test result spectra from Peronema canescens leaf ethanol extract sample**

This phytochemical screening was carried out to qualitatively identify certain secondary metabolite compounds from the ethanolic extract of the leaves of P. canescens (Table 1). Phytochemical screening is also one of the steps of the method used to explore antioxidant compounds in various plants [19]. The results obtained in the phytochemical screening are in line with the exposure of research conducted by Yudha et al. (2021) against the leaves of P. canescens. The study stated that the bioactive components in
P. canescens leaves (alkaloids, flavonoids, terpenoids, steroids, and tannins) are thought to play an important role in certain biological responses of P. canescens plants [21]. The presence of positive flavonoid and tannin compounds is a natural antioxidant with free radical scavenging activity. Natural antioxidant compounds such as flavonoids, phenolic compounds, and other phytochemicals can act as free radical scavengers. These compounds also delay the lipid oxidation process and increase consumer acceptance of food products [22]. These compounds play an important role in preventing chronic disease by slowing the oxidative degradation caused by highly reactive molecules such as reactive oxygen species [23], [24]. Several research results concluded that flavonoid and saponin compounds are closely related to antibacterial, immunomodulatory, or immunostimulant activity. It causes the leaves of P. canescens to be assumed to have great potential for pharmacological activity as herbal medicines to treat various types of disease [25].

FTIR spectroscopy is a high-resolution analytical tool for identifying groups' functional (chemical) constituents and describing compounds structurally. FTIR offers rapid and non-destructive useful group investigation of fingerprint herbal extracts [24]. In other words, the IR spectrum gives a picture of the vibrations of the atoms of a compound. Spectroscopy has developed rapidly due to low noise, fast speed, high replication, relatively straightforward operation, and low cost. FTIR is becoming increasingly valuable for evaluating the quality of herbal ingredients. Most of the molecules show infrared peaks in the middle infrared region (4000–400 cm⁻¹) [26], [27]. Thus, certain submolecular groups produce peaks in specific spectral areas. These characteristic peaks form the empirical basis for interpreting the vibration spectrum. In addition, characteristic absorbance peaks were used to detect (predict) certain compounds related to antioxidant capacity (Figure 3 and Table 2) [28]. Signals from phenolic compounds, which have antioxidant properties, can be found in the 1680–900 cm⁻¹ area. From Table 2, there are six absorbance peaks in this wavelength range. The use of FTIR for the detection of a group of chemical molecules in EELP and other herbal ingredients has provided a fast and sensitive technique with minimal sample preparation that can be performed easily. Then, this method is preferred over other methods for screening chemical compounds in herbal ingredients. It is because the other methods tend to be tiring and time consuming.

The levels of phenolic compounds in the EEPL are shown in Table 4. The content of these phenolic compounds interferes with the mechanism of free radical production either by the formation of chelating reactions on transition metals or by inhibiting the enzymatic reactions involved in the initiation reactions. Phenolic compounds can act as reducing agents, free radical scavengers, hydrogen donors, and inhibitors of pro-oxidative enzymes [2].

Table 5: IC50 antioxidant activity data (DPPH method)

| Test Sample | IC50 (ppm) | 1st iteration | 2nd iteration | Mean |
|-------------|-----------|---------------|---------------|------|
| Dried ethanol extract of Peronema canescens leaves | 116.7747 | 116.7984 | 116.7669 |

Meanwhile, in the results obtained from the high levels of ethanol extract of P. canescens leaves for the total flavonoid content of 142,247 mg QE/g, there is a linear correlation from various studies that the higher the total flavonoid content, the higher the antioxidant capacity [29]. Many epidemiological studies have shown that consuming various leaves of certain plants containing flavonoid and phenolic compounds with strong antioxidant activity are associated with a lower incidence of cardiovascular disease, cancer, diabetes, and neurodegenerative diseases. Flavonoid compounds are secondary metabolites with an antioxidant activity whose potential depends on the number and position of free OH groups. Flavonoid molecules are important antioxidant components responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. They also have characteristic molecular structures ideal for free radical scavenging and have implications for possible therapeutic effects against free radical-mediated diseases [2], [29]. Several flavonoid compounds such as quercetin and catechins are closely related to antioxidant activity and free radical scavengers [2]. Studies on flavonoid compounds and their derivatives have shown various antibacterial, antiviral, anti-inflammatory, and anti-allergic activities [9], [30]. Many reports have shown that daily consumption of high levels of flavonoids can reduce the risk of certain cancers, such as colon, breast, and pancreatic cancer [31], [32], [33].

The test extract was then examined with a set of assays in vitro. Tests were conducted to estimate free radical scavenging activity using the DPPH method [3].
Table 6: Data on antioxidant activity testing (DPPH Method) from Peronema canescens leaf ethanol extract samples at 25°C

| No. | Concentration (ppm) | Absorbance 1st Iteration | Absorbance 2nd Iteration | Percentase Penganhambatan (%) 1st Iteration | Percentase Penganhambatan (%) 2nd Iteration |
|-----|---------------------|--------------------------|--------------------------|--------------------------------------------|--------------------------------------------|
| 1   | 0                   | 0.8289                   | 0.6289                   | 0.0000                                     | 0.0000                                     |
| 2   | 40                  | 0.7008                   | 0.5674                   | 15.4542                                    | 18.2772                                    |
| 3   | 80                  | 0.5056                   | 0.5050                   | 38.9149                                    | 39.0378                                    |
| 4   | 120                 | 0.3391                   | 0.3457                   | 59.0994                                    | 58.2941                                    |
| 5   | 160                 | 0.2464                   | 0.2493                   | 70.2739                                    | 69.9240                                    |
| 6   | 200                 | 0.2009                   | 0.2101                   | 75.7631                                    | 74.6532                                    |

The results of this test can be observed in Tables 5, 6, and Figure 4. The IC50 value from the results of this study is the concentration of the EEPL which can reduce 50% of DPPH free radicals. The smaller the IC50 value, the greater the antioxidant activity of the test sample [34]. The DPPH method is a short and efficient method that is widely used to evaluate antioxidant capacity, which consists of measuring the absorption decay of DPPH levels which are stable free radicals with a purple color. When the free radical scavenger of the ethanolic extract of the leaves of P. canescens was added, DPPH turned yellow due to its conversion to diphenylpicrylhydrazin (DPPH-H) [35]. The IC50 value was calculated from the concentration-response curve in Figure 4. All test analyzes were performed in duplicate.

Conclusion

The results of in vitro research on the ethanolic extract of the leaves of P. canescens found that EEPL has excellent potential as an alternative raw material for products. Some of these products are nutraceutical products, supplements, or phytopharmaca. In this product, the EEPL acts as an antioxidant product. These findings should increase knowledge about the information (data) of the obtained Sungkai leaves (P. canescens).

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