Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jan 06; 10(A):117-124.
https://doi.org/10.3889/oamjms.2022.8221
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Biochemistry*

OPEN ACCESS

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

Tonny Maigoda[1]*, Judiono Judiono[2], Dicki Bakhtiar Purkon[3], Ayu Nala El Muna Haerussana[3], Gurid Pramintarto Eko Mulyo[2]

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

Edited by: Slavica Hristomanova-Mitkovska
Citation: Maigoda T, Judiono J, Purkon DB, Haerussana ANE, Mulyo GPE. Evaluation of *Peronema canescens* Leaves Extract: Fourier Transform Infrared Analysis, Total Phenolic and Flavonoid Content, Antioxidant Capacity, and Radical Scavenger Activity. Open-Access Maced J Med Sci. 2022 Jan 06; 10(A):117-124. https://doi.org/10.3889/oamjms.2022.8221
Keywords: *Peronema canescens* leaves; FTIR analysis; Total phenolic content; Total flavonoid content; Antioxidant activity; DPPH Method
***Correspondence:** Tonny Maigoda, Department of Nutrition, Poltekkes Kemenkes Bengkulu, Bengkulu, Indonesia. E-mail: tonnycmaigoda@yahoo.com
Received: 10-Dec-2021
Revised: 26-Dec-2021
Accepted: 27-Dec-2021

Funding: This study was supported by the BPPSDMK and UPPM Poltekkes Kemenkes Bengkulu, Indonesia
Competing Interest: The authors have declared that no competing interest exists

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, $FeCl_3$ 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 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% $FeCl_3$ 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% $FeCl_3$. 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 H_2SO_4 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 Na_2CO_3 solutions was added to the mixture. The solution was diluted to 25 mL with the addition of distilled H_2O 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:

$$\%\ Inhibition = \left(\frac{Acontrol - Asample}{Acontrol} \right) \times 100$$

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.

Figure 1: Photo of fresh leaf material of Peronema canescens (a), the result of Simplicia from the drying process of fresh leaf material that has previously been washed and chopped (leaf size reduction) (b), and the result of liquid ethanol extract of Peronema canescens leaf (c)

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 2923 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.

Table 2: Comparison of absorbance peaks showing different functional groups in *Peronema canescens* leaf ethanol extract test samples

The functional group (range in cm^{-1})	*Peronema canescens* leaves ethanol extract (in cm^{-1})
N-H bond (amide) (1550–1640)	1600.87
C-N stretch (amine) (1000–1350)	1036.39; 1159.52; 1224.16; 1348.95
C=C stretch (1400–1600)	1454.19
N-H stretch (secondary amine) (3300–3500)	3353.01
C-H stretch (2800–3000)	2923.13
Aliphatic -NH stretching (3100–3500)	3353.01
Aliphatic -CH stretching (2850–3000)	2923.13
-S=O (950–1150)	1036.39
C=C (bending) (665–730)	666.67
O-H stretching (3200–3500)	3353.01
C-H stretching (2850–3000)	2923.13
C-O- stretch (1000–1150)	1036.39
C-O phenol (1050–1260)	1159.52; 1224.16

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.

Table 3: Data on total phenolic levels

No.	Test sample	Total polyphenol content (%)
1.	*Peronema canescens* leaf ethanol extract	5.64

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 Ethanol extract
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 2N+amyl alcohol	Formation of a color change to brownish-yellow	+
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	+
	Aquadest heated+Gelatin 1%	A white precipitate is formed	+
Steroid	Ether/chloroform+Liebermann–Burchard reagent	Formed a blackish-green color	+

+: Positive, −o Negative.

0.618 = 0.0111x+0.3037

0.0111x = 0.618 –0.3037

0.0111x = 0.3146

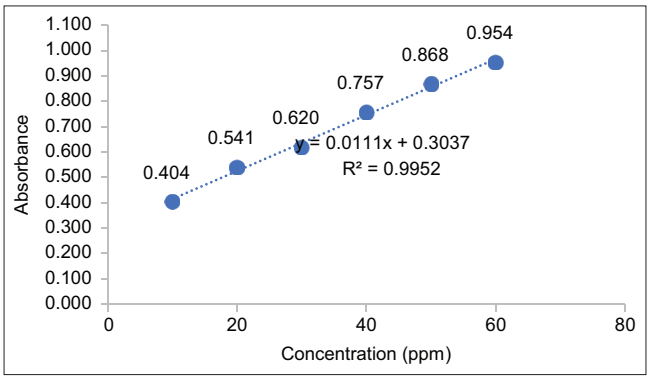
x = 28.4493 ppm

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

= c x v x fp/g

= 142.247 mg QE/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, R2 = 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.



R (linearity)	: 0.9952
a (*intercept*)	: 0.3037
b (*slope*)	: 0.0111
X-bar (mean)	: 0.6908
SD (standard deviation)	: 0.2074
RSD	: 30.0230

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

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

Standard Dilution Absorbance of Quercetin Compound			
Concentration (ppm)	Replication	Absorbance	Mean ± Standard Deviation
10	1	0.400	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	0.869	0.868 ± 0.0006
	2	0.868	
	3	0.868	
60	1	0.956	0.954 ± 0.0015
	2	0.954	
	3	0.953	
The absorbance of *Peronema canescens* leaf extract sample			
Concentration (ppm)	Replication	Absorbance	Mean ± Standard Deviation
Peronema canescens leaf ethanol extract sample	1	0.619	0.618 ± 0.0012
	2	0.619	
	3	0.617	

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].

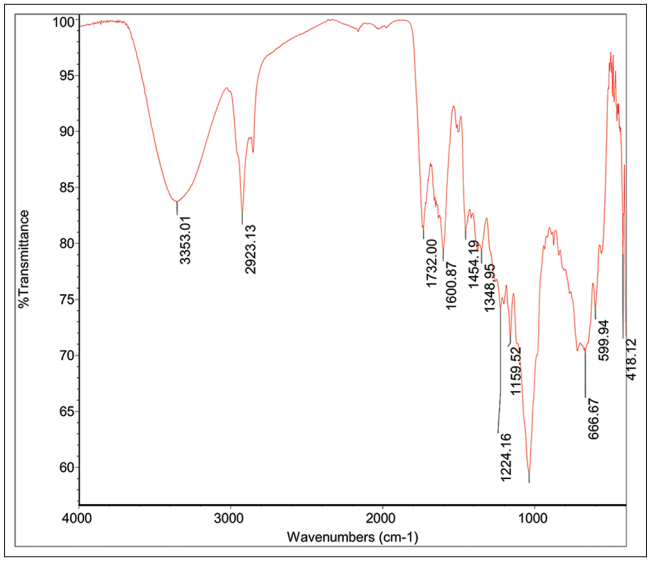


Figure 3: Fourier transform infrared test result spectra from Peronema canescens leaf ethanol extract sample

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].

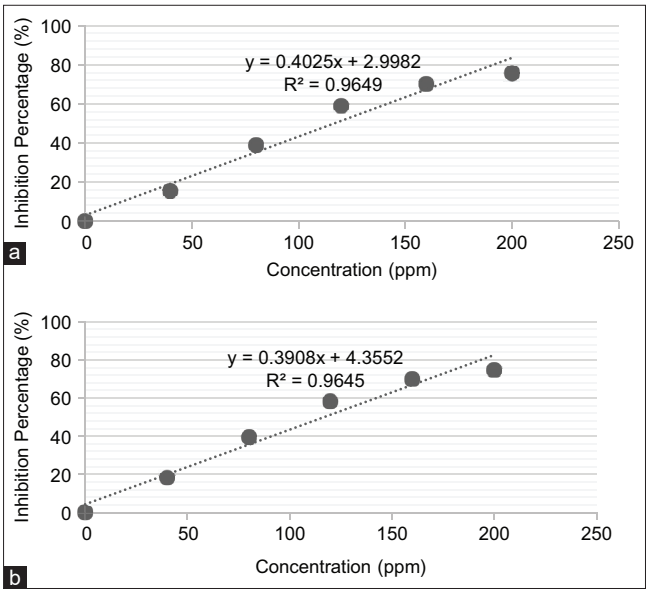


Figure 4: Graphical diagram of antioxidant activity using the DPPH method on samples of dry ethanol extract of Peronema canescens leaves with the 1st repetition (a) and 2nd repetition (b)

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	IC_{50} (ppm)		
	1st iteration	2nd iteration	Mean
Dried ethanol extract of *Peronema canescens* leaves	116.7747	116.7984	116.7865

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		Persentase Penghambatan (%)	
		1st iteration	2nd iteration	1st iteration	2nd iteration
1.	0	0.8289	0.8289	0.0000	0.0000
2.	40	0.7008	0.6774	15.4542	18.2772
3.	80	0.5065	0.5020	38.8949	39.4378
4.	120	0.3391	0.3457	59.0904	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*).

Acknowledgments

The authors acknowledge to the Badan Pengembangan dan Pemberdayaan Sumber Daya Manusia Kesehatan (BPPSDMK) and UPPM Poltekkes Kemenkes Bengkulu which is greatly helped in the process of implementing this research. This research was financially supported by the BPPSDMK and UPPM Poltekkes Kemenkes Bengkulu, Indonesia.

References

1. Nurcholis W, Sya'bani Putri DN, Husnawati H, Aisyah SI, Priosoeryanto BP. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of amomum compactum fruits. Ann Agric Sci. 2021;66(1):58-62. https://doi.org/10.1016/j.aoas.2021.04.001
2. Khalifa HA, Shalaby SI, Abdelaziz AS. Alhagi maurorum aqueous extract protects against norfloxacin-induced hepato-nephrotoxicity in rats. Chinese Herb Med. 2020;12:156-62. https://doi.org/10.1016/j.chmed.2019.09.007
3. Erkekoglou I, Nenadis N, Samara E, Mantzouridou FT. Functional teas from the leaves of arbutus unedo: Phenolic content, antioxidant activity, and detection of efficient radical scavengers. Plant Foods Hum Nutr. 2017;72:176-83. https://doi.org/10.1007/s11130-017-0607-4
   PMid:28421300
4. Putranto AM, Yani AP. Examination of the Sungkai's young leaf extract (*Peronema canescens*) as an antipyretic, immunity, antiplasmodium and teratogenity in mice (*Mus musculus*). Int J Sci Eng. 2014;7:30-4. https://doi.org/10.12777/ijse.7.1.30-34
5. Yani AP, Yenita Y, Ansori I, Irwanto R. The Potential Test of Sungkai Young Leaves (*Peronema canescens*) to Maintain Goodhelth (Immunity) in Mice (*Mus musculus*). Seminar Nasional XI Pendidikan Biologi FKIP UNS; 2013. p. 245-50.
6. Ramadenti F, Sundaryono A, Handayani, D. Test of ethyl acetate fraction of *Peronema canescens* Leaves against *Plasmodium berghei* in *Mus musculus*. Allotrope J Educ Chem. 2017;1:89-92.
7. Ibrahim A, Kuncoro H. Identification of secondary metabolites and antibacterial activity of sungkai leaf extract (*Peronema canescens* Jack.) against several pathogenic bacteria. J Trop Pharm Chem. 2012;2:8-18. https://doi.org/10.25026/jtpc.v2i1.43
8. Hazimah, Teruna HY, Jose C. Antioxidant and antimicrobial activity of *Plectranthus amboinicus* extract. J Farm Indones Res. 2013;1:39-42.
9. Batool S, Saeed H, Yehya S, Ali R, Mofreh A, Saeed H. Evaluation of antidepressant potential of hydrastis canadensis in Mice. Open Access Maced J Med Sci. 2020;8:589-92. https://doi.org/10.3889/oamjms.2020.4903
10. Rahimah SB, Djunaedi DD, Soeroto AY, Bisri T. The phytochemical screening, total phenolic contents and antioxidant activities *in vitro* of white oyster mushroom (*Pleurotus ostreatus*) preparations. Open Access Maced J Med Sci. 2019;7:2404-12. https://doi.org/10.3889/oamjms.2019.741
    PMid:31666837
11. Singh A, Kumar J, Sharma VK, Singh DK, Kumari P, Nishad JH, *et al*. Phytochemical analysis and antimicrobial activity of an endophytic *Fusarium proliferatum* (ACQR8), isolated from a folk medicinal plant *Cissus quadrangularis* L. South Afr J Bot. 2021;140:87-94. https://doi.org/10.1016/j.sajb.2021.03.004.
12. Salem MZ, Ali HM, El-Shanhorey NA, Abdel-Megeed A. Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid contents. Asian Pac J Trop Med. 2013;6(10):785-91. https://doi.org/10.1016/S1995-7645(13)60139-X
    PMid:23870467
13. Sridhar K, Charles AL. *In vitro* antioxidant activity of kyoho grape extracts in DPPH* and ABTS* assays: Estimation methods for EC_{50} using advanced statistical programs. Food Chem. 2019;275:41-9. https://doi.org/10.1016/j.foodchem.2018.09.040
    PMid:30724215
14. Ministry of Health RI. General Standard Parameters of Medicinal Plant Extracts. United States: Ministry of Health RI; 2000.
15. Siregar ES, Pasaribu N, Sofyan MZ. Antioxidant activity of liverworts *Marchantia paleacea* bertol. from North Sumatra, Indonesia. IOP Conf Ser Earth Environ Sci 2021;713:1-7. https://doi.org/10.1088/1755-1315/713/1/012061
16. WHO. Quality Control Methods for Herbal Materials. Geneva: WHO; 2011. p. 283.
17. Kanlayavattanakul M, Pawakongbun T, Lourith N. Dendrobium

orchid polysaccharide extract: Preparation, characterization and *in vivo* skin hydrating efficacy. Chinese Herb Med. 2019;11:400-5. https://doi.org/10.1016/j.chmed.2019.03.012.

18. Fadhilla R, Iskandar AP, Kusumaningrum HD. Antibacterial activity of liver moss plant extract (*Marchantia paleacea*) against pathogenic and food-destroying bacteria. J Technol Food Ind. 2012;23:126-31. https://doi.org/10.6066/jtip.2012.23.2.126
19. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju Y. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal. 2014;22(3):296-302. https://doi.org/10.1016/j.jfda.2013.11.001

    PMid:28911418
20. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of moringa oleifera leaf extract by the appropriate extraction method. Ind Crops Prod. 2013;44:566-71. https://doi.org/10.1016/j.indcrop.2012.09.021
21. Yudha SS, Falahudin A, Wibowo RH, Hendri J, Wicaksono DO. Reduction of 4-nitrophenol mediated by silver nanoparticles synthesized using aqueous leaf extract of *Peronema canescens*. Bull Chem React Eng Catal. 2021;16:253-9. https://doi.org/10.9767/bcrec.16.2.10426.253-259
22. Tohidi B, Rahimmalek M, Arzani A. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of thymus species collected from different regions of Iran. Food Chem. 2017;220:153-61. https://doi.org/10.1016/j.foodchem.2016.09.203

    PMid:27855883
23. Farasat M. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from Northern Coasts of the Persian Gulf. Iran J Pharm Res. 2014;13(1):163-70.

    PMid:24734068
24. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants (Basel). 2017;6(4):42. https://doi.org/10.3390/plants6040042

    PMid:28937585
25. Andespal H, Sundaryono A, Amir H. Phytochemical Profile of Sungkai Leaves (*Peronema canescens*) and Antioxidant Activity Test and Cytotoxic Test Against *Artemia salina* Leach, Undergraduated Thesis. Indonesia: Universitas Bengkulu; 2020.
26. Joshi DD. Herbal Drugs and Fingerprints: Evidence-Based Herbal Drugs. Berlin, Germany: Springer; 2012. p. 121-46. https://doi.org/10.1007/978-81-322-0804-4
27. Khare B, Mishra MK, Kesharwani L. Screening of adulterants in slimming herbal formulation by FTIR analysis. Eur J Med Plants. 2019;30(2):1-8. https://doi.org/10.9734/ejmp/2019/v30i230174
28. Silva S, Feliciano RP, Boas LV, Bronze MR. Application of FTIR-ATR to moscatel dessert wines for prediction of total phenolic and flavonoid contents and antioxidant capacity. Food Chem. 2014;150:489-93. https://doi.org/10.1016/j.foodchem.2013.11.028

    PMid:24360480
29. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala, N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants (Basel). 2019;8(4):96. https://doi.org/10.3390/plants8040096

    PMid:30978964
30. Saeed N, Khan MR, Shabbir, M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med. 2012;12:221. https://doi.org/10.1186/1472-6882-12-221

    PMid:23153304
31. Hossain MA, AL-Raqmi KA, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pac J Trop Biomed. 2013;3(9):705-10. https://doi.org/10.1016/S2221-1691(13)60142-2

    PMid:23998010
32. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, *et al*. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015;20(12):21138-56. https://doi.org/10.3390/molecules201219753

    PMid:26633317
33. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L.-A phytochemical and pharmacological review. Food Chem. 2014;165:424-43. https://doi.org/10.1016/j.foodchem.2014.05.002

    PMid:25038696
34. Offermanns S, Rosenthal W. Encyclopedia of Molecular Pharmacology. 2nd ed. Berlin, Germany: Springer; 2008.
35. López-Munguía A, Hernández-Romero Y, Pedraza-Chaverri J, Miranda-Molina A, Regla I, Martínez A, Castillo E. phenylpropanoid glycoside analogues: Enzymatic synthesis, antioxidant activity and theoretical study of their free radical scavenger mechanism. PLoS One. 2011;6(6):e20115. https://doi.org/10.1371/journal.pone.0020115

    PMid:21674039