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POTENTIAL ANTIOXIDANT ACTIVITY OF SURUHAN LEAF AND STEM EXTRACT (*Peperomia pellucida* L. Kunth) USING THE DPPH METHOD AND DETERMINATION OF PHENOLIC AND FLAVONOID LEVELS

Jajang Japar Sodik[1], Wempi Budiana[1*], Asep Roni[1], Wahyudin[1]
[1]*Fakultas Farmasi, Universitas Bhakti Kencana Universitas Bhakti Kencana, Bandung 40614, Indonesia.*
**Email Corresponding: wempi.budiana@bku.ac.id*

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ABSTRACT

Antioxidants are compounds that are capable of neutralizing free radicals. One plant with potential antioxidant properties is *Peperomia pellucida.* **Objective:** This study aimed to determine the antioxidant activity, total phenolic content, and total flavonoid content of extracts of the leaves and stems of *Peperomia pellucida.* **Methods:** Antioxidant activity was tested using the DPPH method, while the total phenolic and flavonoid contents were measured using UV-Vis spectrophotometry. Extraction was performed via reflux using three different solvents: n-hexane, ethyl acetate, and ethanol. **Results:** The highest antioxidant activity was found in the ethanol extract of the leaves, with an IC_{50} value of 36.85 µg/mL, and in the stems, with an IC_{50} value of 71.28 µg/mL. The highest total phenolic content in the ethanol extract of the leaves was 14.46 mg GAE/g and that in the stems was 12.82 mg GAE/g. The highest total flavonoid content in the ethanol extract of the leaves was 12.12 mg QE/g and that in the stems was 10.49 mg QE/g. **Conclusion:** The results indicated that the ethanol extract of *Peperomia pellucida* leaves exhibited very strong antioxidant activity.

Keywords: *antioxidants, DPPH, phenolic, flavonoids, suruhan Plants*

INTRODUCTION

Free radicals are molecules with one or more unpaired electrons that are highly reactive towards electrons in the body. These molecules can damage biomolecules by disrupting DNA, proteins, and lipid integrity, thereby increasing oxidative stress, which plays a role in the development of degenerative diseases such as hypertension and diabetes mellitus (Phaniendra *et al*., 2015). Sources of free radicals include food coloring, preservatives, dust, air pollution, cigarette smoke, and UV radiation. Antioxidants inhibit the formation of free radicals in the body. Poor diet and lifestyle can increase the excessive production of free radicals, which are harmful to health (Tristantini *et al*., 2016).

Antioxidants are biological molecules that can reduce or eliminate the harmful effects of oxidants in the body by donating an electron to them, thereby inhibiting their activity. Antioxidants can be categorized as endogenous, such as glutathione and ubiquinone produced by the body, and exogenous, such as vitamins C, E, and beta-carotene obtained from food (Rao and Møller, 2011).

Peperomia pellucida (L.) Kunth, known as "shiny bush," is an herbaceous plant of the *Piperaceae* family that thrives in loose, humid soil under the shade of trees (Alves *et al*., 2019). In traditional medicine, across many pantropical countries, *Peperomia pellucida* is used to treat various ailments. In Malaysia, this plant is considered a vegetable that can be boiled to treat rheumatism, fatigue, bone pain, and muscle aches (Alves *et al*. 2019). *Peperomia pellucida* contains bioactive compounds, such as alkaloids, flavonoids, sterols,

tannins, reducing sugars, amino acids, saponins, triterpenoids, carbohydrates, phenols, azulenes, carotenoids, depsides, and quinones, which have immunomodulatory, antibacterial, anti-inflammatory, anti-glycemic, and analgesic potential (Alves *et al*., 2019; Ooi *et al*., 2012). The antioxidant activity of *Peperomia pellucida* can suppress oxidative stress in various metabolic diseases (Ng *et al*., 2020).

This study aimed to determine the antioxidant activity, total phenolic content, and total flavonoid content of extracts of the leaves and stems of *Peperomia pellucida*. Antioxidant activity was tested using the DPPH method, while the total phenolic and flavonoid contents were measured using UV-Vis spectrophotometry.

RESEARCH METHODS

The research methodology included several stages: material preparation, crude drug characterization, extraction, phytochemical screening, antioxidant activity testing using the DPPH method, and determination of phenolic and flavonoid content.

Material Preparation:

This stage involved the collection of materials, plant determination, and material processing.

Crude drug characterization

Characterization involved determining the total ash content, acid-insoluble ash content, ethanol-soluble extract content, water-soluble extract content, moisture content, and drying loss.

Extraction:

The extraction was performed using a stepwise reflux extraction method with 1000 mL of n-hexane, 1000 mL of ethyl acetate, and 1000 mL of ethanol at a temperature of 60°C. The extract was then concentrated using a rotary evaporator to obtain a concentrated extract.

Phytochemical Screening:

Phytochemical screening includes testing for the presence of alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids.

Antioxidant Activity Testing:

Qualitative antioxidant activity was tested using thin-layer chromatography (TLC), with vitamin C as the comparator and 0.2% DPPH in methanol as the spot indicator. Antioxidant activity is visually indicated by yellow spots against a purple background on the plate and is stable for 30 minutes.

Quantitative antioxidant activity testing was performed using the DPPH scavenging method and UV-Vis spectrophotometry. The DPPH solution was mixed with the test solution at a 1:1 ratio, and the absorbance was measured at 515 nm after incubation for 30 minutes at room temperature.

Determination of the Total Phenolic Content:

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method with gallic acid as the standard. The results are expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of extract.

Determination of the Total Flavonoid Content:

The total flavonoid content was determined using the Chang method with quercetin as the standard. Results were expressed as milligrams of quercetin equivalents (QE) per 100 grams of extract.

RESULTS AND DISCUSSION

Characterization of Crude Drugs

The characterization of simplicia is conducted to ensure that the simplicia used in this research meets high-quality standards and is suitable for use. Additionally, this characterization is essential to ensure reproducibility, as the standard of the samples used is clearly defined (Sari, 2015).

Table I. Characterization Results of Suruhan Leaves and Stems

Examination	Leaf Results (% w/w)	Stem Results (% w/w)	Standard Farmakope Herbal Indonesia
Total ash content	10	8,5	< 2,6
Ash insoluble in acid	5	6,5	< 0,04
Ethanol-soluble extractives	27,5	25	>24,3
Water-soluble extractives	22,5	10	>24,6
Drying shrinkage	5,98	7,90	< 10
Moisture content	5*	7,5*	10

Note: * = % v/w

Physicochemical parameters, such as Total Ash Content, Acid Insoluble Ash Content, Ethanol Soluble Extract Content, Water Soluble Extract Content, Loss on Drying, and Moisture Content, are presented in Table I. Moisture content is an inevitable component of crude drugs that should be minimized as much as possible. The moisture content of 5% in the leaves and 7.5% in the stems was not excessively high, thus not facilitating the growth of microorganisms. A total ash content of 10% in leaves and 8.5% in stems, and an acid-insoluble ash content of 5% in leaves and 6.5% in stems indicate the presence of overall foreign inorganic matter. The ash value represents inorganic salts present in the drug.

These results provide insight into the characterization of *Peperomia pellucida* leaves and stems, which has not been previously performed. Previous studies on plant materials from Indonesia showed a moisture content of 9.68%, with total ash, water-soluble ash, and acid-insoluble ash values not exceeding 7.03%, 5.27%, and 0.55% w/w, respectively (Hanani *et al*., 2017). Another study on *Peperomia pellucida* plant material from Malaysia revealed a high total ash content (31.22%), indicating a high-value mineral composition, mainly comprising potassium, calcium, and iron as the primary elements, with a moisture content of 8.33% (Ooi *et al*., 2012).

Phytochemical Screening

Phytochemical screening was conducted to identify the secondary metabolite groups present in the leaves and stems of suruhan. The results of phytochemical screening can be used as a reference to determine the compounds responsible for antioxidant activity.

Table II. Phytochemical Screening Results

Compound Groups	Simplicia		Extracts					
			N-hexane		Ethyl Acetate		Ethanol 96%	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Quinon	-	-	-	-	-	-	-	-
Saponins	+	+	-	-	-	-	+	+
Steroids/ Triterpenoids	+	+	+	+	+	+	+	+

Explanation:

\+ Presence of the compound group tested

\- Absence of the compound group tested

Extraction

The leaves and stems of P. pellucida were extracted using the reflux method. Reflux extraction is a hot extraction method in which plant material is soaked in a solvent and then heated to facilitate the transfer of compounds from the leaves and stems to the solvent. The reflux method was selected to ensure maximal and efficient extraction of the desired compounds, aided by the application of external heat energy.

The extraction process was carried out using three different solvents with varying polarities: ethanol, ethyl acetate, and n-hexane, with 100 grams of the sample used for each extraction. This was done to ensure that all compounds present in the leaves and stems of *Peperomia pellucida* could be fully extracted using each appropriate solvent.

Once n-hexane, ethyl acetate, and ethanol extracts were obtained, antioxidant activity testing was performed by comparing the antioxidant properties of the three test extracts. This comparison helped to identify the extract that exhibited better antioxidant properties. Furthermore, it allows for the estimation of the compounds responsible for this activity.

Table III. Extract Yield

Sample	Extracts Results % b/b	Extracts Stem % b/b
N-Hexane	4,79	3,01
Ethyl Acetate	6,67	3,65
Ethanol	8,07	4,01

Extract Monitoring

Extract monitoring aims to determine the presence of active compounds with antioxidant activity, flavonoid compounds, and phenolic compounds in the extract. The extracts were monitored using thin-layer chromatography (TLC) on a silica gel GF254 stationary phase and eluted with three different mobile phases: n-hexane: ethyl acetate (7:3) as non-polar, chloroform: methanol (9:1) as semi-polar, and butanol: acetic acid: water (4:1:5) as polar. This extract monitoring system utilized four visualizing reagents: 10% H_2SO_4 in methanol as a universal visualizing reagent, 10% $FeCl_3$ in methanol for phenolic compounds, 5% $AlCl_3$ for flavonoid compounds, and 0.2% DPPH in methanol for antioxidant compounds, with vitamin C as a reference.

A. Non-polar

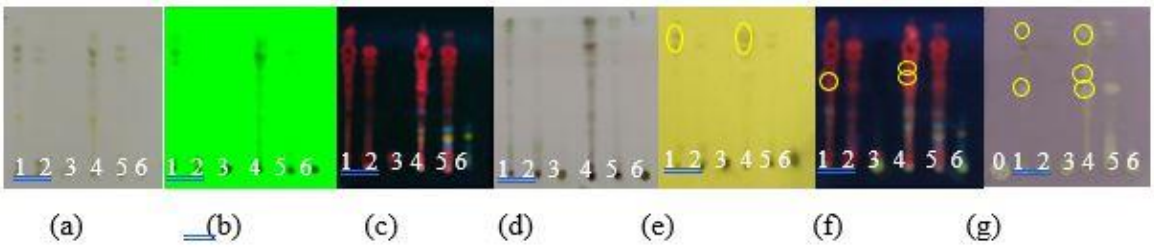

Figure 1. Chromatogram results of the n-hexane extract with n-hexane: ethyl acetate (7:3) mobile phase, stem extract (1, 2, 3), leaf extract (4, 5, 6), and vitamin C standard (0). Visual spot appearance (a), UV 254 nm (b), UV 366 nm (c), H_2SO_4 10% in methanol (d), $FeCl_3$ 10% in methanol (e), $AlCl_3$ under UV 366 nm (f), and DPPH 0.2% in methanol (g).

The results showed that the DPPH antioxidant runs parallel to the phenol group indicated by the $FeCl_3$ spot and runs parallel to the flavonoid indicated by the $AlCl_3$ spot. It is possible that the antioxidant activity of this extract is due to phenolic and flavonoid compounds

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B. Semi Polar

In these results, the DPPH antioxidant aligns with the phenolate group observed in the $FeCl_3$ spot and correlates with the flavonoid spot observed in the $AlCl_3$ visualization. This suggests that the antioxidant activity of this extract may be due to phenolate and flavonoid compounds.

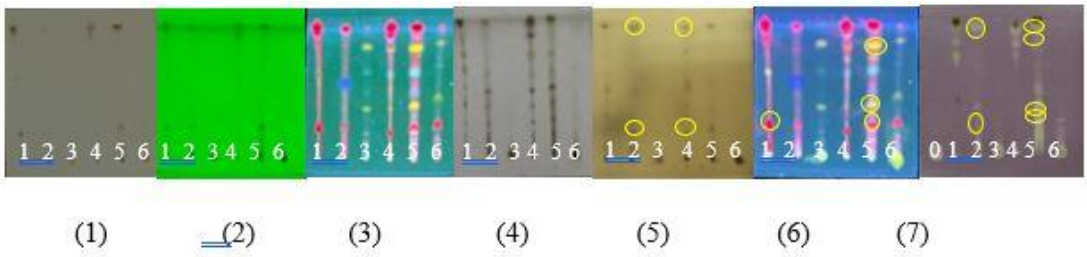

Figure 2. Chromatogram results of the ethyl acetate extract using the mobile phase chloroform: methanol (9:1). Stem extract (1, 2, 3), leaf extract (4, 5, 6), and vitamin C standards (0). Visual spot appearance (1), UV 254 nm (b), UV 366 nm (c), 10% H_2SO_4 in methanol (d), 10% $FeCl_3$ in methanol (e), $AlCl_3$ observed under UV 366 nm (f), and 0.2% DPPH in methanol (g).

C. Polar

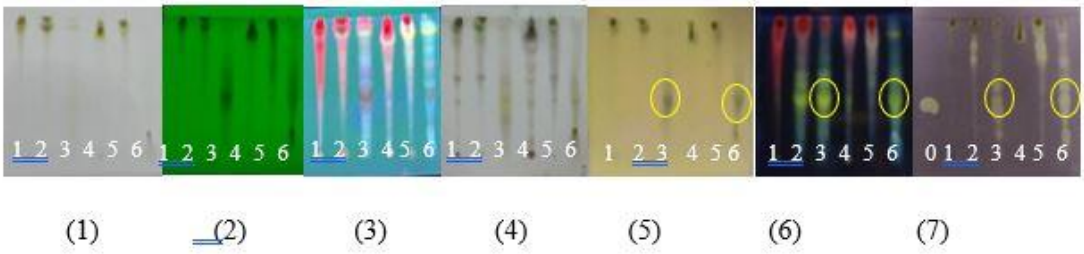

Figure 3. Chromatogram results of 96% ethanol extract with mobile phase butanol : acetic acid : water (4:1:5). Stem extract (1.2.3), leaf extract (4,5,6), and vitamin C comparator (0). Visual spot appearance (1), UV 254 nm (b), UV 366 nm (c), 10% H_2SO_4 in methanol (d), 10% $FeCl_3$ in methanol (e), $AlCl_3$ under UV light at 366 nm (f), and 0.2% DPPH in methanol (g).

These results indicate that the DPPH antioxidant aligns with the phenolate compound indicated by the $FeCl_3$ spot and with the flavonoid compound indicated by the $AlCl_3$ spot. This suggests that the antioxidant activity of this extract was likely due to phenolate and flavonoid compounds. The use of three different developing agents aims to determine the separation of each solvent with different polarities, allowing us to identify suitable solvent separation based on their polarity.

Quantitative Test of DPPH Antioxidant Activity

Antioxidant activity testing involved construction of a calibration curve for the DPPH solution to demonstrate the linear relationship between the absorbance response of the solution and the concentration of the DPPH solution. Optimization was carried out in the wavelength range 400–800 nm. The DPPH wavelength obtained had a maximum absorption of 515 nm in methanol. Subsequently, absorbance measurements were performed at this wavelength.

The linear regression equation obtained from the DPPH calibration curve was $y = 0.0103x + 0.1576$ with a coefficient of determination (R^2) of 0.9977. Based on the above calibration curve, it can be used to perform antioxidant testing in vitro on extract samples.

The absorbance of both the test solution and the control solution were within the linearity range of the prepared DPPH calibration curve.

Antioxidant activity testing on leaf and stem extracts of suruhan was conducted at λ = 515 nm, which is the maximum wavelength of DPPH used. Subsequently, the IC_{50} value for the test sample was calculated using a linear regression equation (y = bx + a). Y represents the 50% free radical scavenging value. Where Y is the IC_{50} of the test sample. The IC_{50} value indicates the effective concentration that can reduce 50% of DPPH free radicals. The smaller the IC_{50} value, the higher the antioxidant activity.

Vitamin C was used as a reference for antioxidant activity testing. Vitamin C was selected because of its strong antioxidant activity. Vitamin C was incubated with 70 ppm DPPH in a 1:1 ratio for 30 minutes and absorbance was measured at a wavelength of 515 nm. The % inhibition values for each concentration series were obtained from the measurements, and a curve and its equation were constructed.

Table IV. Results of Antioxidant Activity Testing

Pelarut	IC_{50} (µg/mL)	
	***Peperomia pellucida* leaves**	***Peperomia pellucida* Stem**
n-Hexane	101,69 ± 0,21	140,51 ± 0,34
Ethyl Acetate	72,60 ± 0,30	82,81 ± 0,18
Ethanol 96 %	36,85 ± 0,11	71,28 ± 0,10
Vitamin C	7,90 ± 0,01	7,90 ± 0,01

Note: IC_{50} = Concentration required to reduce 50% of DPPH free radicals

Based on the results of the quantitative antioxidant activity test of the extract samples using the DPPH method, it showed very strong IC_{50} values for the leaves of the suruhan, namely in the 96% ethanol extract, with 36.85 µg/mL (very strong). Meanwhile, the strongest result for the suruhan stem was found in the 71.28 µg/mL ethanol extract (strong).

The positive control used as a positive C because vitamin C has an excellent ability to neutralize free radicals and is a good source of antioxidants. In addition, vitamin C has high polarity due to its many hydroxyl groups, making it easier to reduce free radicals. The difference in IC_{50} values between each extract and the vitamin C comparator was due to the ability of each compound to donate electrons to DPPH. The more electrons given to DPPH, the lower its absorbance value, which means an increase in the inhibition percentage and a decrease in the IC_{50} value to IC_{50} 7.90 (Very Strong). Uwaya *et al*. (2021) reported that both aqueous and methanol extracts of the plant exhibited in vitro antioxidant scavenging activities, as evidenced by multiple assays, such as DPPH, ABTS, and hydrogen peroxide scavenging activities (Ibe-Diala and Igwe, 2022; Uwaya *et al*., 2021). Similarly, Almira *et al*. (2023) highlighted the potent antioxidant activity of the ethanolic extract and its fractions, with the highest flavonoid content observed in the ethanolic extract, correlating with the strong antioxidant efficacy. Ibe-Diala and Igwe (2022) also confirmed the presence of phytochemicals, such as alkaloids, phenols, and flavonoids, in the leaves of plants, which contribute to its significant antioxidant activity. The antioxidant effects of Peperomia pellucida can be attributed to the presence of bioactive compounds such as flavonoids, phenolics, and alkaloids (Ibe-Diala and Igwe, 2022; Uwaya *et al*., 2021). These compounds neutralize free radicals, thereby preventing oxidative stress and cellular damage. Flavonoids, for instance, can donate hydrogen atoms to free radicals, stabilize them, and stop the chain reaction of lipid peroxidation. Phenolics possess hydroxyl groups that can react with free radicals to form stable phenoxy radicals that do not initiate further oxidation. Alkaloids may also contribute to antioxidant activity through various mechanisms including metal chelation and radical scavenging (Ibe-Diala and Igwe, 2022; Uwaya *et al*., 2021).

Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu method. The reaction involved a reduction-oxidation reaction. Phenolic compounds reduce phosphomolybdate-phosphotungstate in the Folin-Ciocalteu reagent, forming blue-colored molybdenum.

Table V. Total Phenolic Content Test Results

Total Phenolic Content % (mg GAE/g Extract)		
Sample	***Peperomia pellucida* leaves**	***Peperomia pellucida* Stem**
n-Hexane	10.01 ± 0.03	9.52 ± 0.04
Ethyl acetate	12.64 ± 0.04	11.96 ± 0.07
Ethanol 96%	14.46 ± 0.05	12.82 ± 0.03

The research results indicated that the highest total phenolic content was found in the ethanol 96% extract of suruhan leaves, which was 14.46% (mg GAE/g Extract). The highest total phenolic content in suruhan stems was found in the ethanol 96% extract, which was 12.82% (mg GAE/g Extract).

Total Flavonoid Content

The total flavonoid content of the extracts was determined using the Chang method. This technique uses 2% AlCl3 in ethanol, which is then incubated with the sample. Colorimetry using aluminum chloride involves the formation of a stable complex between aluminum chloride and keto hydroxy and ortho-dihydroxy groups. Therefore, aluminum chloride can be used to detect both groups of flavonoid molecules.

Table VI. Total Flavonoid Content Test Results

Total Flavonoid Content % (mg QE/ g Extract)		
Sample	***Peperomia pellucida* leaves**	***Peperomia pellucida* Stem**
n-Hexane	7,52 ± 0,03	6,88 ± 0,07
Ethyl acetate	10,41 ± 0,05	9,02 ± 0,05
Ethanol 96%	12,12 ± 0,04	10,49 ± 0,03

The results indicated that the highest total flavonoid content in *Peperomia pellucida* leaves was in the 96% ethanol extract at 12.12% (mg QE/g Extract). Meanwhile, the highest total flavonoid content in *Peperomia pellucida* stems was in the 96% ethanol extract at 10.49% (mg QE/g Extract).

CONCLUSION

The research concluded that *Peperomia pellucida* L. Kunth demonstrated antioxidant activity through qualitative screening using TLC. Yellow spots against a purple background after DPPH reagent spraying were observed for all six extracts. The 96% ethanol extract of the leaves had the lowest IC_{50} value of 36.85 µg/mL, indicating very strong antioxidant activity. The stem extract showed strong antioxidant activity, with an IC_{50} value of 71.28 µg/mL in the 96% ethanol extract. The highest total flavonoid content was observed in the leaves of the 96% ethanol extract at 12.12% (mg QE/g Extract), and the highest total phenolic content was observed in the same extract at 14.46% (mg GAE/g Extract).

Peperomia pellucida exhibits significant antioxidant activity, particularly in the 96% ethanol leaf extract, indicating its potential as a strong natural antioxidant. This finding has implications for the development of health supplements and antioxidant-based cosmetics. Future research should explore antioxidant mechanisms, isolate and characterize bioactive compounds, and conduct clinical trials to ensure safety and efficacy in humans.

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