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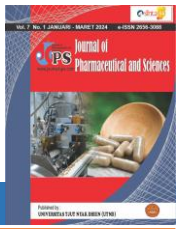

Phytochemical Screening, GC-MS Analysis, and Antioxidant Activity (DPPH Method) of Ethanol Extract of Chinese Betel Leaf (*Peperomia Pellucida* (L.) Kunth)

Skrining Fitokimia, Analisis GC-MS, dan Aktivitas Antioksidan (Metode DPPH) Ekstrak Etanol Daun Sirih Cina (*Peperomia Pellucida (L.) Kunth*)

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Abstract

Introduction: *Peperomia pellucida* (L.) Kunth, commonly known as Chinese betel leaf, is a medicinal plant widely used in traditional medicine for various ailments. The plant is reported to contain diverse secondary metabolites with potential pharmacological activities, including antioxidant properties. However, comprehensive information on its phytochemical constituents and antioxidant potential remains limited, particularly for extracts of Indonesian origin. **Objective:** This study aimed to determine the phytochemical profile, chemical constituents, and antioxidant activity of the ethanol extract of *P. pellucida* leaves. **Methods:** Dried leaf powder (100 g) was extracted by maceration using 96% ethanol (1:10 w/v) for 3 × 24 hours at room temperature. Phytochemical screening was conducted using standard qualitative tests for alkaloids, flavonoids, glycosides, saponins, steroids/terpenoids, and tannins. Chemical composition was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, with IC_{50} values determined by linear regression analysis. Vitamin C served as the positive control. **Results:** The extraction yielded a concentrated ethanol extract of 11.8% (w/w). Phytochemical screening revealed the presence of flavonoids, glycosides, saponins, and terpenoids/steroids, while alkaloids and tannins were not detected. GC-MS analysis identified 14 chromatographic peaks, with major compounds including methyl hexadecanoate (14.12%), methyl 9-octadecenoate (13.61%), and triacontanoic acid. The ethanol extract exhibited very strong antioxidant activity with an IC_{50} value of 41.47 µg/mL, compared to vitamin C (IC_{50} = 4.083 µg/mL). The linear regression equation for the extract was y = 0.4291x + 32.2016 (R^2 = 0.9985). **Conclusion:** The ethanol extract of *P. pellucida* leaves contains various secondary metabolites and demonstrates very strong antioxidant activity. Although GC-MS analysis was predominantly characterized by fatty acid esters, the observed bioactivity likely results from synergistic contributions of multiple bioactive constituents. Further studies employing LC-MS/MS are recommended to identify specific compounds responsible for the antioxidant activity and to quantitatively determine total phenolic and flavonoid contents to establish their correlation with the observed bioactivity.

Keywords*: *Peperomia pellucida, phytochemical screening, GC-MS, DPPH antioxidant.

Abstrak

Pendahuluan: *Peperomia pellucida* (L.) Kunth atau daun sirih Cina merupakan tanaman obat yang banyak digunakan dalam pengobatan tradisional. Tanaman ini dilaporkan mengandung berbagai metabolit sekunder dengan aktivitas farmakologis yang potensial, termasuk sebagai antioksidan. Namun demikian, informasi yang komprehensif mengenai profil fitokimia dan aktivitas antioksidannya masih terbatas, khususnya untuk ekstrak yang berasal dari Indonesia. **Tujuan:** Penelitian ini bertujuan untuk menentukan profil fitokimia, kandungan senyawa kimia, dan aktivitas antioksidan ekstrak etanol daun *P. pellucida*. **Metode:** Serbuk daun kering (100 g) diekstraksi dengan metode maserasi menggunakan etanol 96% (1:10 b/v) selama 3 × 24 jam pada suhu ruang. Skrining fitokimia dilakukan menggunakan uji kualitatif standar untuk alkaloid, flavonoid, glikosida, saponin, steroid/terpenoid, dan tanin. Komposisi kimia dianalisis menggunakan kromatografi Gas-Spektrometri massa (GC-MS). Aktivitas antioksidan dievaluasi menggunakan metode peredaman radikal 2,2-difenil-1-pikrilhidrazil (DPPH), dengan nilai IC_{50} ditentukan melalui analisis regresi linear. Vitamin C digunakan sebagai kontrol positif. **Hasil:** Ekstraksi menghasilkan rendemen ekstrak etanol pekat sebesar 11,8% (b/b). Skrining fitokimia menunjukkan adanya flavonoid, glikosida, saponin, dan terpenoid/steroid, sedangkan alkaloid dan tanin tidak terdeteksi. Analisis GC-MS mengidentifikasi 14 puncak kromatogram, dengan senyawa utama meliputi metil heksadekanoat (14,12%), metil 9-oktadekenoat (13,61%), dan asam triakontanoat. Ekstrak etanol menunjukkan aktivitas antioksidan yang sangat kuat dengan nilai IC_{50} sebesar 41,47 µg/mL, dibandingkan dengan vitamin C (IC_{50} = 4,083 µg/mL). Persamaan regresi linier untuk ekstrak adalah y = 0,4291x + 32,2016 (R^2 = 0,9985). **Kesimpulan:** Ekstrak etanol daun *P. pellucida* mengandung berbagai metabolit sekunder dan menunjukkan aktivitas antioksidan yang sangat kuat. Meskipun analisis GC-MS didominasi oleh ester asam lemak, aktivitas bioaktif yang teramati kemungkinan merupakan hasil kontribusi sinergis dari berbagai konstituen bioaktif. Penelitian lanjutan menggunakan LC-MS/MS direkomendasikan untuk mengidentifikasi senyawa spesifik yang bertanggung jawab atas aktivitas antioksidan, serta menentukan kuantitatif kadar fenolik dan flavonoid total untuk menetapkan korelasinya dengan aktivitas bioaktif yang teramati.

Kata Kunci: Peperomia pellucida, skrining fitokimia, GC-MS, antioksidan DPPH.

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Introduction

Indonesia is known for its high biodiversity, with around 30,000-40,000 plant species, of which 2,500-7,500 are used as medicinal plants (Ministry of Health of the Republic of Indonesia, 2020). The many historical uses of these plants as medicinal treatments may be attributed to their bioactive compounds, which exhibit a wide array of pharmacological effects, including antioxidant, antibacterial, and anti-inflammatory activities [1]. Antioxidant activity stands out among them because of its connection to warding off free radical-induced degenerative illnesses.

Exposure to free radicals is increasing in society due to various environmental factors, including air pollution, cigarette smoke, and ultraviolet radiation. Heart disease, stroke, and cancer are just a few degenerative illnesses that an excess of free radicals may trigger. These molecules can destroy biological molecules, including lipids, proteins, and DNA [2]. The human body has natural antioxidants. However, they are not sufficient to deal with all the free radicals in the body, so antioxidants from natural sources are needed [3]. Despite their low molecular weights, antioxidant compounds may inhibit oxidation by blocking the generation of radicals [4].

Phytochemical components, including tannins, alkaloids, and flavonoids, are often found in plants with antioxidant activity [5]. For example, Chinese betel leaf (*Peperomia pellucida* (L.) Kunth), a popular herbal remedy for gastrointestinal issues, acne, and headaches, is said to possess such properties [2].

Using phytochemical screening techniques and GC-MS to investigate the chemical compounds present in this plant, this study aims to determine the secondary metabolite content of Chinese betel leaves. The initial step of this study is to perform extraction using the maceration method [6]. One compound separation method that involves soaking in an organic solvent at a specific temperature is known as maceration. Not only is it simple and cheap to accomplish, but the pressure differential between the cell's interior and exterior also causes its walls and membranes to rupture. Consequently, the solvent will dissolve the secondary metabolites found in the cytoplasm [7]. The GC-MS method uses mass spectrometry to analyze substances. If the sample contains volatile solutes, they may be separated using gas chromatography. An appropriate procedure is used to create ions from inorganic or organic compounds. Then, the ions of a compound are separated according to their mass-to-charge ratio (m/z). Finally, the abundance and m/z of each compound are used to detect ions qualitatively and quantitatively [8].

The GC-MS method has several advantages, including efficiency and high resolution, which allow it to analyze very small particles. The gas flow is highly controlled, and its speed remains constant. Fast analysis, usually only a few minutes. Does not damage the sample. Extremely sensitive; can evaluate a wide range of substances at low concentrations; and can distinguish molecules that are in solution [8]. Finding chemicals that readily evaporate under low pressure and high vacuum is the goal of gas chromatography. Mass spectrometry addresses the molecular formula, molecular weight, and production of charged molecules [9].

In this study, GC-MS was used to identify the types of compounds present in Chinese betel leaves, particularly volatile compounds, which are expected to contribute to the activity of their extract. It was decided to use the DPPH antioxidant activity assay. The purpose of this research was to collect detailed information in light of the description given above. The combination of these methods is expected to provide a comprehensive understanding, with phytochemical screening serving as initial detection of compound groups. In contrast, GC-MS provides more specific compound identification [10].

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Methods

This study employed an experimental method using *Peperomia pellucida* (L.) Kunth leaves as the test material. The study was conducted from 2024 to 2025 at the Integrated Laboratory of the Islamic University of Indonesia, the Madanense Herbarium (MEDA), University of North Sumatra, and the Research Laboratory of the Faculty of Pharmacy, Tjut Nyak Dhien University, Medan. Purposive sampling was used.

A total of 100 g of dried simplicia powder of *Peperomia pellucida* leaves was macerated with 1 L of 96% ethanol (1:10 w/v) for 3 × 24 h at room temperature. The mixture was stirred every 6 h to enhance solvent penetration and extraction efficiency. The solvent was replaced every 24 h, and all filtrates were combined and concentrated under reduced pressure at 50 °C using a rotary evaporator to obtain a viscous ethanol extract.

Phytochemical screening was conducted to identify the secondary metabolites present in the ethanol extract of *P. pellucida*. The investigated compounds included alkaloids, glycosides, triterpenoids, saponins, steroids, and flavonoids. Chemical constituents of the extract were further identified using Gas Chromatography–Mass Spectrometry (GC-MS).

Antioxidant Assay Using the DPPH Method

Antioxidant activity was evaluated using the DPPH radical scavenging assay. A DPPH solution at 40 ppm was prepared in ethanol and used as the working solution. For the antioxidant test, 100 µL of sample solution at concentrations of 5, 10, 25, 50, and 100 µg/mL was mixed with 100 µL of DPPH solution (40 ppm). The mixtures were incubated for 20 minutes at room temperature in the dark. Absorbance was measured at a wavelength of 515 nm using a UV–Vis spectrophotometer. Vitamin C was used as the positive control. Vitamin C solutions were prepared at concentrations of 2, 4, 6, 8, and 10 µg/mL and treated under the same conditions as the sample solutions.

The percentage inhibition of DPPH radicals was calculated using the following equation:

$$\%\ Inhibition\ =\ [(Acontrol\ -\ Asample)\ /\ Acontrol]\ \times\ 100$$

where *Acontrol* is the absorbance of the control solution, and *Asample* is the absorbance of the sample solution.

The IC_{50} value was determined using linear regression analysis between concentration (x) and percentage inhibition (y), expressed by the equation:

$$y\ =\ ax\ +\ b$$

The IC_{50} value was calculated using:

$$IC_{50}\ =\ (50\ -\ b)/a$$

where a is the slope and b is the intercept of the regression equation. The coefficient of determination (R^2) was used to evaluate the goodness of fit of the regression model.

Calculation of Yield

A total of 5 kg of fresh *Peperomia pellucida* leaves yielded 130 g of dried simplicia powder, corresponding to a simplicia yield of 2.6%. Subsequent extraction of 100 g of simplicia powder with 96% ethanol yielded 11.8 g of concentrated extract, for an extract yield of 11.8%.

Table 1. **A**ntioxidant Activity of Ethanol Extract of *Peperomia pellucida*

Sample	Regression equation	R^2	IC_{50} (µg/mL)	Category
Ethanol extract of *Peperomia pellucida*	y=0.4291x 32.2016	0.9985	41.47	Very strong

The linear regression analysis of the ethanol extract produced the equation **y =** 0.4291x + 32.2016 (R^2 = 0.9985)**,** resulting in an IC_{50} value of 41.47 µg/mL**.**

Table 2. Vitamin C

Sample	Regression equation	R^2	IC_{50} (µg/mL)	Category
Vitamin	y=5.3563x + 28.2183	0.9937	4.083	Very strong

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The linear regression analysis of vitamin C produced the equation y = 5.3563x + 28.2183 (R^2 = 0.9937), resulting in an IC_{50} value of 4.083 µg/mL.

Results and Discussion

Table 3. Phytochemical Screening Results of EEDSC

No.	Screening	Reagents	Conclusion
1.	Alkaloid	Mayer	(-)
		Wagner	(-)
		Dragendroff	(-)
		Bouchardart	(-)
2.	Flavonoid	FeCl3 5%	(-)
		NaOH 10%	(-)
		Mg + HCl (p)	(+)
		H2SO4 (p)	(-)
3.	Terpenoid/Steroid	Salkowsky	(-)
		Liberman-buchar	(+)
4.	Saponin	HCl + hot water (shaken)	(+)
5.	Tannin	FeCl3 1%	(-)
6.	Glycoside	Acetic Acid + Sulfuric Acid	(+)

Information:
EEDSC: Ethanol Extract of Chinese Betel Leaf
(+) : Contains compound
(-) : Does not contain a compound

A total of 5 kg of fresh *Peperomia pellucida* leaves yielded 130 g of dried simplicia powder, corresponding to a simplicia yield of 2.6%. Maceration of 100 g of simplicia powder using 96% ethanol produced 11.8 g of concentrated extract, corresponding to an extract yield of 11.8%. Phytochemical screening revealed the presence of glycosides, terpenoids/steroids, saponins, and flavonoids in the ethanol extract of *P. pellucida*.

Extraction methods using ethanol as a solvent have proven effective for extracting compounds across a wide range of polarities, from polar to semi-polar/less polar, allowing the dissolution of both lipophilic and semi-polar/polar compounds [11]. This is reinforced by results from phytochemical screenings in various studies, which show that ethanol extracts can isolate flavonoids, phenolics, tannins, saponins, terpenoids, and even less polar compounds. Therefore, the use of ethanol as a solvent supports the robustness of plant extracts as a source of bioactive secondary metabolites. As a moderately polar solvent, ethanol can extract various classes of compounds, including flavonoids, phenolics, fatty acids, and their ester derivatives.

According to Table 3 above, the phytochemical examination results for glycosides, terpenoids/steroids, flavonoids, and saponins in the ethanol extract of Chinese betel leaves are favorable.

GC-MS EEDSC Analysis Results

Using a retention time range of 14-33 minutes, GC-MS analysis of an ethanol extract of Chinese betel leaves (*Peperomia pellucida* (L.) Kunth) obtained from the UII Yogyakarta Laboratory revealed a complex phytochemical profile. The presence of prominent chemicals in the extract is indicated by three primary peaks with retention periods of 16.193 minutes (14.22%), 16.032 minutes (13.61%), and 14.291 minutes (14.01%). There is a high degree of certainty in identifying the bulk of the chemicals, which are fatty acid esters, primarily methyl palmitate and methyl oleate. The 14 peaks detected in the chromatogram, each representing volatile or semi-volatile compounds separated in the chromatography system, were then analyzed by mass spectrometry, as seen in Figure 1.

Although the GC-MS profile was dominated by fatty acid esters and other primary metabolites, the observed antioxidant activity may result from the combined contribution of both primary and secondary metabolites present in the extract. Phytochemical screening confirmed the presence of flavonoids, glycosides, saponins, and terpenoids/steroids. However, these compounds were not clearly detected by GC-MS because many secondary metabolites are polar, thermolabile, and non-volatile, making them unsuitable for direct GC-MS analysis. Therefore, more advanced analytical techniques such as LC-MS/MS are required to confirm and characterize the specific bioactive compounds responsible for the antioxidant activity of *P. pellucida*. In

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addition, several minor peaks observed in the chromatogram could not be confidently identified because their similarity index values were below 50%, and thus no specific compound assignment was made.

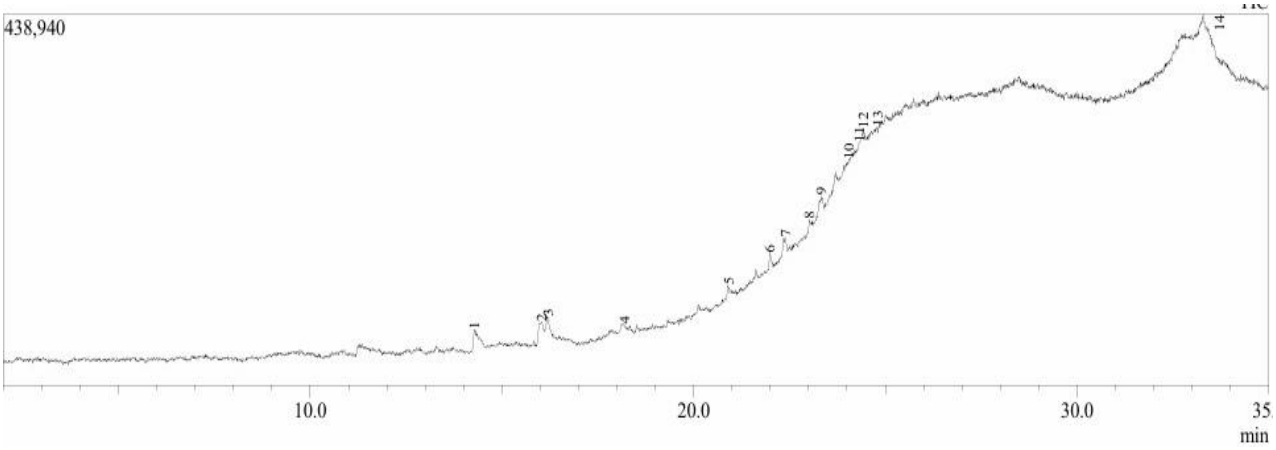

Figure 1. Total Ion Chromatogram (TIC) of Ethanol Extract of *Peperomia pellucida*

Table 4. Major Compounds Identified by GC-MS in Ethanol Extract of *Peperomia pellucida*

Peak No.	Retention Time (min)	Compound Identified	Molecular Formula	Molecular Weight (g/mol)	Area (%)	Compound Class
1	14.291	Methyl Hexadecanoate (Methyl Palmitate)	$C_{17}H_{34}O_2$	270.45	12.10	Fatty acid ester
2	16.032	9-Octadecenoic Acid Methyl Ester (Methyl Oleate)	$C_{19}H_{36}O_2$	296.49	13.61	Fatty acid ester
3	16.193	Octadecanoic Acid Methyl Ester (Methyl Stearate)	$C_{19}H_{38}O_2$	298.51	14.12	Fatty acid ester

As shown in Table 4, three major compounds were identified in the ethanol extract of *Peperomia pellucida*, namely methyl stearate (14.12%), methyl oleate (13.61%), and methyl palmitate (12.10%). These compounds belong to the fatty acid ester group and represent the dominant constituents detected by GC-MS analysis.

Table 5. GC Fragmentation Results

Peak#	R. Time	1.Time	F.Time	Area	Area%
1	14.291	14.208	14.482	167198	12.10
2	16.032	15.917	16.092	187947	13.61
3	16.193	16.092	16.308	195004	14.12
4	18. 180	18. 033	18. 283	46170	3.34
5	20.899	20. 858	20. 958	29481	2.13
6	21.995	21.933	22.075	63725	4.61
7	22.387	22.300	22.433	102053	7.39
8	23.017	22.983	23.258	90150	6.53
9	23.334	23.258	23.408	121921	8.83
10	24. 050	24. 033	24. 283	70202	5.08
11	24. 317	24. 283	24. 375	60942	4.41
12	24. 416	24. 375	24. 433	63015	4.56
13	24. 788	24.483	24.808	130086	9.42
14	33. 292	33.183	33.392	53505	3.87
Total				1381399	100.00

Among all the peaks, several are dominant by peak area, indicating higher compound concentrations. The largest peak, covering 19,5004 square pixels, or about 14.12% of the entire area, occurs at a retention time of 16.193 minutes. This indicates that the compound at this peak is the major component in the extract. Other

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large peaks appear at retention times of 16.032 minutes (13.61%) and 14.291 minutes (12.10%), which are also compounds with high content in the sample.

The mass spectrometry of each peak shows that several identified compounds appear in the retention time range of 14.2 to 16.1 minutes, including methyl hexadecanoic acid, 9-octadecenoic acid, and methyl ester known as methyl palmitate. These compounds are esters of palmitate acid and oleate acid, which are saturated fatty acids esterified with a methyl group and unsaturated fatty acids [12]. These compounds are widely known for their biological roles, including antibacterial activity, antioxidant properties, and potential applications in the pharmaceutical and cosmetic fields [13].

In addition to methyl palmitate, several other compounds were also identified with moderate to high similarity, including octadecanoic acid (stearate), linoleic acid, tetradecanoic acid (myristate), and triacontanoic acid. These compounds exhibit variations in carbon chain structure and degree of saturation, thereby enriching the phytochemical profile of the Chinese betel leaf extract. Some of these compounds are classified as weak unsaturated acids [14]. Overall, the Chinese betel leaf extract, extracted with ethanol, shows a phytochemical profile dominated by fatty acid esters.

Based on the GC-MS analysis, the identified compounds were predominantly primary metabolites, especially fatty acid esters and long-chain fatty acids. Primarily occurring compounds in plant metabolism include methyl esters of essential fatty acids such as linoleic acid (9,12-octadecadienoic acid), stearic acid (octadecanoic acid), and oleic acid (9-octadecenoic acid), which are themselves derivatives of these acids.

Three main chemicals were identified in the GC-MS study of the Chinese betel leaf ethanol extract: methyl hexadecanoate (14.12%), methyl 9-octadecenoate (13.61%), and triacontanoic acid, which was detected as one of the predominant long-chain fatty acids in the extract [15,16].

Flavonoids, steroids, and saponins were among the beneficial secondary metabolites found by phytochemical screening. However, specific compounds from these groups were not detected as major components in the GC-MS chromatogram. Several minor peaks with long retention times were observed, but their similarity index values were below 50%, so they could not be identified with confidence. Several minor peaks with long retention times were observed in the chromatogram; however, these peaks could not be confidently identified because their similarity index values were below 50%. Therefore, no specific compound assignment was made, and further analysis with more sensitive analytical techniques is required for compound identification [17,18].

Several technical factors can explain the discrepancy between the results of phytochemical screening and the GC-MS profile. First, GC-MS has limitations in analyzing polar, thermolabile compounds and large-molecular-weight compounds, such as flavonoid glycosides and oxygenated lignans. These compounds tend to degrade at high temperatures within the GC column or are not sufficiently volatile to be carried by the mobile gas phase [8]. Second, the abundance of secondary metabolites in the extract is likely much lower than that of primary metabolites. Peaks of secondary metabolites can be masked or co-eluted with the highly dominant peaks of primary metabolites, making their detection and identification suboptimal. Third, the derivatization process (if not performed) can also affect the detection of polar compounds. Although derivatization was not performed in this study, 96% ethanol as a solvent can extract compounds with a wide range of polarities, including semi-polar compounds that may not be ideal for direct GC-MS analysis [17]. Click or tap here to enter text. Another possible explanation is that a defatting step was not performed before extraction and GC-MS analysis. Consequently, fatty acids and their methyl ester derivatives remained abundant in the extract and may have dominated the chromatographic profile. Future studies are recommended to include a defatting step with n-hexane before extraction or to employ sequential extraction (n-hexane, ethyl acetate, and ethanol) to separate compounds by polarity and improve detection of antioxidant-related secondary metabolites.

Thus, a GC-MS profile dominated by fatty acids does not necessarily exclude the presence of other bioactive compounds that were not optimally detected by this analytical method. On the contrary, these results indicate that obtaining a comprehensive secondary metabolite profile of Peperomia pellucida requires a different analytical approach, such as LC-MS/MS, which is better suited to polar, non-volatile compounds. LC-MS/MS can separate and identify polar and non-volatile compounds more effectively than GC-MS and may provide a more comprehensive characterization of the secondary metabolites present in *Peperomia pellucida* extract [18,19]. Although these compounds were not conclusively identified in this GC-MS analysis, this is likely due to their low concentration, co-elution, or thermal degradation during analysis. The strong antioxidant activity of the extract (IC_{50} 41.47 µg/mL) suggests that multiple bioactive constituents may contribute to its free radical scavenging activity. However, the specific compounds responsible for this activity

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could not be conclusively identified in the present study. One limitation of this study is the absence of quantitative determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC).

Although phytochemical screening confirmed the presence of flavonoids and other secondary metabolites, their concentrations were not quantitatively measured. Therefore, the contribution of phenolic and flavonoid compounds to the observed antioxidant activity could not be quantitatively assessed. Future studies are recommended to determine TPC and TFC values and evaluate their correlation with antioxidant activity. Thus, the obtained GC-MS profile still provides a comprehensive picture of the major components of the extract and can serve as a basis for further studies, such as LC-MS/MS analysis, extract standardization, or the development of natural-based products.

Another limitation of this study is that antioxidant measurements were performed without biological replication and statistical comparison between treatment groups. Consequently, variability among measurements could not be estimated through standard deviation analysis or inferential statistical tests such as ANOVA or t-test. Future studies should perform measurements in triplicate and include appropriate statistical analyses to improve the reliability and reproducibility of the results.

Results of Antioxidant Activity Testing

First, the DPPH standard stock solution has to be prepared for the antioxidant activity test. Then, the maximum wavelength needs to be determined. Next, the reaction time (operating time) is measured to determine the duration required for a compound to react with another to form a stable product. To determine the IC50, we first need to know the assay duration. Then, we measure the absorbance of DPPH upon reacting with the ethanol extract of Chinese betel leaf (Peperomia pellucida (L.) Kunth).

After adding the test sample, the DPPH absorbance was measured to determine the antioxidant activity of the ethanol extract of Chinese betel leaf (Peperomia pellucida (L.) Kunth). This means the sample can neutralize or scavenge DPPH radicals. A decrease in DPPH absorbance indicates antioxidant activity; the magnitude of the decline is proportional to the strength of this effect.

Measurement results of the maximum wavelength of DPPH

Utilizing a UV-VIS spectrophotometer, the highest absorbance of an ethanol solution containing 40 ppm of DPPH was quantified. The absorbance of 0.449 at 515 nm was the highest for the DPPH solution in ethanol, according to the measurements. This wavelength falls within the working range of the UV-Vis spectrophotometer, 400–800 nm. In the present study, absorbance measurements were performed once for each concentration level without analytical replication. Therefore, the reported values represent single measurements, and no standard deviation could be calculated.

Measurement results of operating time

The purpose of determining the operating time is to identify the optimal time at which the solution reaches stability, indicated by the absence of a decrease in absorbance. Measurement results show that the operating time for the DPPH solution is stable between the 6th and 7th minutes and the 20th and 21st minutes.

IC_{50} value analysis results

Table 6. Data on the Antioxidant Activity Results of the Ethanol Extract of Chinese Betel Leaves.

Sample	Sample Concentration (µg/mL)	Absorbance	% Damping	IC_{50}
Chinese Betel Leaf Ethanol Extract	5	0,298	33,63	41,47 µg/mL
	10	0,282	37,19	
	25	0,254	43,42	
	50	0,211	53,00	
	100	0,111	75,27	

Table 6 shows that at doses of 5, 10, 25, 50, and 100 ppm, the absorbance values decreased. This is because the ethanol extract solution from Chinese betel leaves has antioxidant properties. The level of antioxidant activity is directly proportional to the absorbance value. A value of 41.47 µg/mL was found in the IC_{50} calculation, suggesting that the ethanol extract of Chinese betel leaves has antioxidant activity that is classified as "very strong." According to Molyneux (2004), antioxidant activity is classified as very strong when

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the IC_{50} value is below 50 µg/mL. [20], An IC_{50} value below 50 µg/mL indicates that the antioxidant is particularly potent.

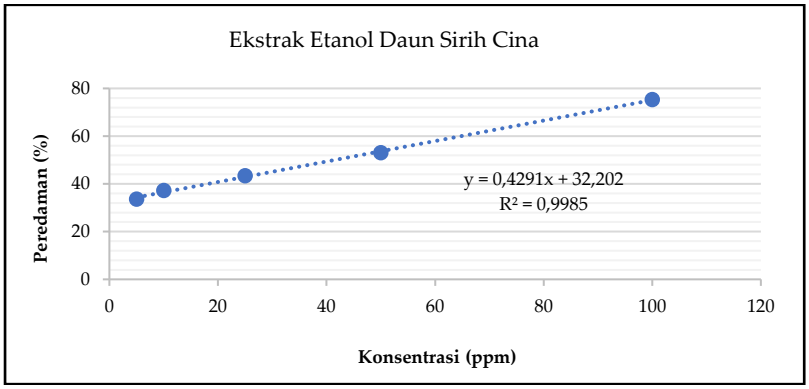

Figure 2. Linear Regression Curve for Determination of IC_{50} Value of Ethanol Extract of Peperomia pellucida Using the DPPH Method

According to the antioxidant classification proposed by Molyneux (2004), extracts with IC_{50} values below 50 µg/mL are categorized as having very strong antioxidant activity. Therefore, the ethanol extract of *P. pellucida* in this study can be classified as a very strong antioxidant source [20].

The ethanol extract of Chinese betel leaves exhibited significant antioxidant activity (IC_{50} = 41.47 µg/mL), consistent with the phytochemical screening results indicating the presence of flavonoids, terpenoids/steroids, glycosides, and saponins. Although the GC-MS profile was dominated by fatty acid esters, the observed antioxidant activity may result from the combined contribution of various bioactive compounds present in the extract. Because GC-MS has limitations in detecting polar, thermolabile, and non-volatile compounds, the specific constituents responsible for the antioxidant activity could not be conclusively identified in this study [4,21]. Therefore, further analysis using LC-MS/MS or bioassay-guided fractionation is recommended to identify and characterize the major antioxidant compounds of *Peperomia pellucida* extract [22].

The dominance of fatty acids in the GC-MS profile does not mean that fatty acids do not contribute to antioxidant activity. Several studies have shown that unsaturated fatty acids, such as linoleic acid and oleic acid, which are recognized by their methyl esters, also exhibit antioxidant properties, even if their processes may not be as strong as those of phenolic compounds [16,23]. However, the specific compounds responsible for the observed antioxidant activity could not be conclusively identified in this study. The antioxidant activity is likely attributable to the combined contributions of various bioactive constituents present in the extract. Therefore, further analysis using LC-MS/MS or bioassay-guided fractionation is recommended to identify the major antioxidant compounds of *Peperomia pellucida* [24]. In other words, the measurable antioxidant activity is the result of the cooperative (synergistic) effect of all bioactive components in the extract, not just a single compound. Future metabolomic approaches or bioassay-guided fractionation are required to definitively identify the compounds responsible for the plant's primary antioxidant activity. The specific compounds responsible for the antioxidant activity could not be conclusively identified in this study. Therefore, further analysis using LC-MS/MS or bioassay-guided fractionation is recommended to identify the major antioxidant constituents of *Peperomia pellucida* extract.

The antioxidant activity observed in this study (IC_{50} = 41.47 µg/mL) is comparable to that reported in previous studies on *Peperomia pellucida*. Yanti et al. (2023) reported IC_{50} values of 24.51 µg/mL and 30.50 µg/mL for 70% and 80% ethanol extracts of *P. pellucida*, respectively, which were lower than the IC_{50} value obtained in the present study. Variations in antioxidant activity may be attributed to differences in ethanol concentration, the geographical origin of the plant material, the harvesting period, environmental conditions, extraction procedures, and the phytochemical composition of the extract. These factors may affect the concentration and profile of bioactive compounds responsible for antioxidant activity [25].

Furthermore, Anggreni et al. (2023) demonstrated that gummy candy formulated with *P. pellucida* extract retained considerable antioxidant activity, indicating that the bioactive compounds remain stable after formulation processing[26]. A review by Widyantari and Sari (2023) also summarized numerous studies showing that *P. pellucida* consistently exhibits moderate to very strong antioxidant activity depending on extraction method, solvent polarity, plant origin, and phytochemical composition [27]. Differences in IC_{50}

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values among studies may be influenced by geographical origin, harvesting time, environmental conditions, extraction techniques, solvent systems, and analytical procedures employed during antioxidant testing [28], [29].

Recent studies have further highlighted the antioxidant potential of *P. pellucida*. Phong et al. (2024) reported that extraction solvents significantly influence the extraction yield, phytochemical composition, and antioxidant activity of *P. pellucida*, suggesting that solvent polarity is critical for recovering antioxidant constituents [28]. In addition, Tuan and Men (2024) reviewed the phytochemical composition and antioxidant properties of *P. pellucida*. They concluded that flavonoids, phenolic compounds, and other secondary metabolites are major contributors to its biological activities [29]. These findings support the present study, where phytochemical screening confirmed the presence of flavonoids and other bioactive compounds in the ethanol extract.

Table 7. Data on the Results of Vitamin C Antioxidant Activity

Sample	Sample Concentration (µg/mL)	Absorbance	%Damping	IC_{50}
Vitamin C	2	0,278	38,08	4,083 µg/mL
	4	0,219	51,22	
	6	0,177	60,57	
	8	0,138	69,26	
	10	0,078	82,62	

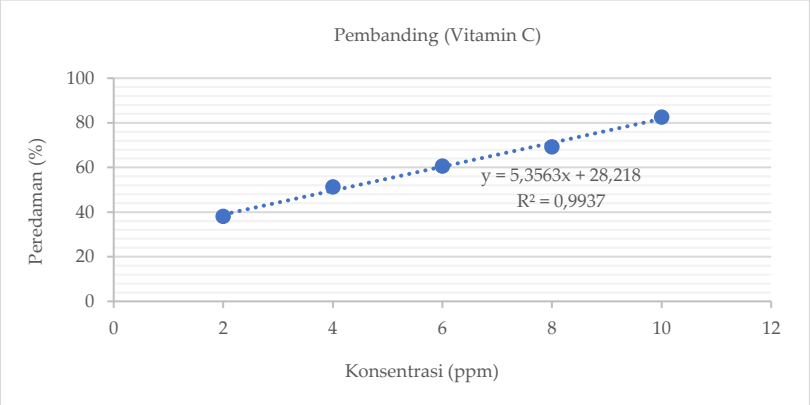

Figure 3. Linear Regression Curve for Determination of IC_{50} Value of Vitamin C Using the DPPH Method

As shown in Table 7, the absorbance values decreased as vitamin C concentration increased from 2 to 10 µg/mL, indicating enhanced radical-scavenging activity. Lower absorbance values reflect greater antioxidant activity due to the reduction of DPPH radicals. Vitamin C exhibited a very strong antioxidant activity with an IC_{50} value of 4.083 µg/mL. This result is consistent with previous studies reporting an IC_{50} value of 4.2539 µg/mL for vitamin C, which is also classified as a very strong antioxidant according to the criteria proposed by Molyneux (2004) [21].

Conclusions

The ethanol extract of *Peperomia pellucida* (L.) Kunth leaves contained flavonoids, glycosides, saponins, and terpenoids/steroids as confirmed by phytochemical screening. GC-MS analysis revealed that the extract was predominantly composed of fatty acid esters, with methyl hexadecanoate, methyl 9-octadecenoate, and octadecanoic acid methyl ester identified as the major constituents. The extract exhibited very strong antioxidant activity with an IC_{50} value of 41.47 µg/mL in the DPPH assay. These findings suggest that *P. pellucida* leaves represent a promising natural source of antioxidant compounds. Further studies employing LC-MS/MS and quantitative analysis of phenolic and flavonoid contents are recommended to identify the specific compounds responsible for the observed antioxidant activity.

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Conflict of Interest

The authors declare that they have no known competing financial interests, personal relationships, or affiliations that could have appeared to influence the work reported in this manuscript.

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