

## The Antioxidant Activity of Ethanol Extract of Chinese Betel Leaf (*Peperomia pellucida*) using the DPPH Method with Potential as an Antidiabetic Agent

Fahmi Sadik<sup>1,\*</sup>, Mahgial Anwar<sup>1</sup>, Muh Nasir<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Biology, Pharmacy Study Program, Faculty of Medicine and Health Sciences, Khairun University, Ternate, Indonesia.

<sup>2</sup>Department of Pharmaceutical Chemistry, Pharmacy Study Program, Faculty of Medicine and Health Sciences, Khairun University, Ternate, Indonesia.

\*e-mail correspondence: [fahmisadik@unkhair.ac.id](mailto:fahmisadik@unkhair.ac.id)

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Complications of diabetes mellitus are known to be exacerbated by oxidative stress. The Chinese pepper plant (*Peperomia pellucida*), known as a source of natural antioxidants, was studied for its potential in this research. We evaluated the in vitro antioxidant capacity of the ethanol extract of *P. pellucida* leaves using the DPPH method to see its relationship with antidiabetic potential. The process involves extracting the leaves using ethanol, followed by testing the extract's ability to scavenge DPPH free radicals at various concentration levels to determine the IC<sub>50</sub> value. It was found that the ethanol extract showed antioxidant activity with an IC<sub>50</sub> of 65.813 ppm, indicating its effectiveness as a free radical scavenger. This study concludes that the ethanol extract of Chinese betel leaves has strong in vitro antioxidant activity based on the DPPH test. These results support the further development of this extract as an adjunct therapy for diabetes mellitus, primarily in reducing oxidative stress. Follow-up suggestions include testing more specific antidiabetic mechanisms (such as  $\alpha$ -amylase/ $\alpha$ -glucosidase inhibition and in vivo studies), fractionation to isolate active compounds, and toxicity evaluation for development safety

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### INTRODUCTION

Diabetes mellitus (DM) is a persistent metabolic condition with an increasing prevalence worldwide [1]. Oxidative stress, resulting from an imbalance between the formation of free radicals and the body's antioxidant defense capabilities, is a critical factor in the etiology of diabetes complications [2]. Therefore, antioxidant compounds capable of neutralizing free radicals are essential to help reduce the negative effects of oxidative stress and improve blood glucose control.

Although antidiabetic drugs are widely available, their use is often limited by potential long-term side effects, including the risk of hypoglycemia, digestive problems, and the development of insulin resistance [3-4]. Furthermore, the majority of these synthetic drugs are not designed to protect against oxidative stress. In contrast, medicinal plants rich in bioactive compounds such as flavonoids, tannins, and various phenolic compounds offer potential as safer

and more cost-effective antioxidant and antidiabetic agents [5]. This phenomenon has led to increased interest among researchers in exploring the potential of local flora as an alternative therapeutic source.

One plant that has garnered attention is *Peperomia pellucida*, commonly known as Chinese betel leaf. This plant grows wild and has been empirically used by local communities to address various health concerns, such as inflammation, wound healing, and metabolic disorders [6]. Preliminary studies have identified antioxidant compounds in the leaves of Chinese betel, including flavonoids, saponins, and phenolic acids, which are believed to contribute to its ability to inhibit oxidation processes [7]. In line with this, research by Hidayati et al. (2023) also indicates the potential of this plant in lowering blood glucose levels, further strengthening its position as a promising antidiabetic agent candidate [8].

Although the traditional use of Chinese betel leaf is well known, in-depth quantitative studies on the antioxidant activity of its extract, particularly those using ethanol as a solvent and tested with the DPPH method, have been limited. The DPPH method itself is a standard analytical technique in laboratories for evaluating the capacity of an extract or compound to neutralize free radicals [9-10]. The lack of quantitative data justifies the need for further research on the antioxidant potential of Chinese betel leaf.

Thus, this study focuses on evaluating the ability of ethanol extract from *Peperomia pellucida* leaves to combat free radicals, measured using the DPPH method. Additionally, this research aims to assess the correlation between this activity and its potential antidiabetic effects. The results of this study are expected to provide a strong scientific basis for the development of complementary treatments using local medicinal plants. Such therapy is anticipated to be safe, cost-effective, and beneficial in combating oxidative stress and aiding in diabetes management.

## MATERIALS AND METHODS

This study employs a laboratory experimental approach to examine the capacity of ethanol extract from Chinese betel leaf (*P. pellucida*), collected from Ternate Island in North Maluku, to neutralize free radicals. The activity was tested using the Diphenyl-picrylhydrazyl (DPPH) reagent with 96% ethanol as the solvent. A series of laboratory equipment was used in this research, including a measuring cylinder, volumetric flask, dropper pipette, cuvette, analytical balance, test tubes, micropipette, micro-tube, oven, UV-Vis spectrophotometer, spatula, and vortex mixer.

### Preparation of Chinese Betel Leaf Sample

A total of 300 grams of fresh Chinese betel leaf (*P. pellucida*) was cleaned with running water and then pulverized. This wet sample was then dried in an oven at 50°C for 24 hours. The resulting dry simplisia was ground into coarse powder using a blender and sifted using a mesh sieve with a size of 40 to obtain a homogeneous particle size powder [11].

### Extraction of Chinese Betel Leaf Sample

Extraction was performed on 50 grams of Chinese betel leaf powder (*P. pellucida*) using the maceration method. A total of 500 mL of 96% ethanol was used as the solvent. The maceration process was carried out in a closed glass container for 3 days (72 hours) at room temperature with periodic shaking. After the maceration period, the mixture was filtered to separate the liquid extract (filtrate) from the solid residue.

### Preparation of DPPH Solution

The preparation of the DPPH solution began by accurately weighing 4 mg of DPPH powder. This powder was first dissolved in analytical-grade ethanol using a beaker and then quantitatively transferred to a 100 mL volumetric flask. After stirring until homogeneous, the solution volume was adjusted to the 100 mL mark by adding more analytical-grade ethanol [12].

### Determination of Maximum Absorption Wavelength of DPPH

To achieve optimal measurement sensitivity, the maximum absorption wavelength ( $\lambda_{\text{max}}$ ) of DPPH was identified. This process involved using a UV-Vis spectrophotometer to scan the absorption of DPPH in the spectrum range from 400 to 800 nm [13].

### Preparation of Test Extract Solution

A stock solution of Chinese betel leaf extract (*P. pellucida*) was first prepared at a concentration of 1000 ppm. To do so, 100 mg of dried extract was weighed, dissolved in analytical-grade ethanol, and homogenized. This solution was then transferred to a 100 mL volumetric flask, and the volume was adjusted to the 100 mL mark with the addition of analytical-grade ethanol. From this 1000 ppm stock solution, test solutions with concentrations of 60, 50, 40, 30, and 20 ppm were prepared. This process involved pipetting precise volumes of the stock solution (0.6, 0.5, 0.4, 0.3, and 0.2 mL) into separate 10 mL volumetric flasks. Each solution was then diluted with analytical-grade ethanol to the 10 mL mark.

### Preparation of Vitamin C Comparison Solution

As a positive control in the testing, a standard Vitamin C solution was also prepared. This involved weighing 10 mg of standard Vitamin C, which was then dissolved in ethanol and quantitatively transferred to a 100 mL volumetric flask. The final solution volume was adjusted to the 100 mL mark by adding 96% ethanol, yielding a 100 ppm Vitamin C stock solution.

### Antioxidant Activity Test with DPPH

Antioxidant activity was tested by pipetting the Chinese betel leaf extract solutions (20, 30, 40, 50, and 60 ppm) and Vitamin C solution (10, 15, 20, 25, and 30 ppm) as a positive control into separate test tubes. A total of 2 mL of DPPH solution was then added to each tube, followed by homogenization using a vortex mixer. The reaction mixture was incubated for 30 minutes at room temperature and in the dark (without light exposure). After the incubation period, the absorbance of each solution was measured at the  $\lambda_{\text{max}}$  of DPPH (517 nm) using a UV-Vis spectrophotometer.

### IC<sub>50</sub> Calculation

The IC<sub>50</sub> value was calculated using the equation derived,  $Y = bx + a$ . The IC<sub>50</sub> parameter value was then calculated using Eq. 1:

$$IC_{50} = \frac{50 - a}{b} \quad (1)$$

## RESULTS AND DISCUSSION

The extraction of *Peperomia pellucida* leaf simplisia was performed using the maceration method with 96% ethanol as the solvent. The maceration method was selected for its simplicity and effectiveness in extracting bioactive compounds without compromising their chemical structure, particularly for heat-sensitive compounds. After a 3 × 24-hour soaking and filtration process, the solvent was evaporated to obtain a concentrated extract. The yield of the 96% ethanol extract was 12.264%, indicating that a considerable amount of active compounds was successfully extracted and concentrated into a thick extract.

This yield is higher compared to a study by Dharma Yanti et al., 2023, which reported yields of 2.48% for *Peperomia pellucida* leaf extract using 70% ethanol and 1.6% using 80% ethanol. The difference in yields may be attributed to the varying concentrations of solvent used, with 96% ethanol having a greater capacity to dissolve nonpolar and semi-polar compounds found in *Peperomia pellucida* leaves, such as flavonoids, alkaloids, and terpenoids. Additionally, factors such as the extraction method, maceration time, particle size of the simplisia, and the solvent evaporation technique could also influence the extraction efficiency and yield. Generally, higher

concentrations of ethanol result in the extraction of more active compounds, as long as the compounds are compatible with the solvent.

The  $IC_{50}$  value, representing the concentration required to achieve 50% inhibition, was calculated for each sample using the obtained regression equation. The results showed that the  $IC_{50}$  value for Vitamin C was 1.454 ppm (Table 1), while for the *Peperomia pellucida* leaf extract, it was 65.813 ppm (Table 2). This significant difference in values suggests that the antioxidant potential of Vitamin C is far superior to that of the *Peperomia pellucida* leaf extract in this assay system. Furthermore, the steeper slope of the regression curve for Vitamin C (0.4124) compared to that of the *Peperomia pellucida* leaf extract (0.2150) visually supports this conclusion, indicating that the increase in Vitamin C concentration results in a more pronounced inhibition effect than the test extract.

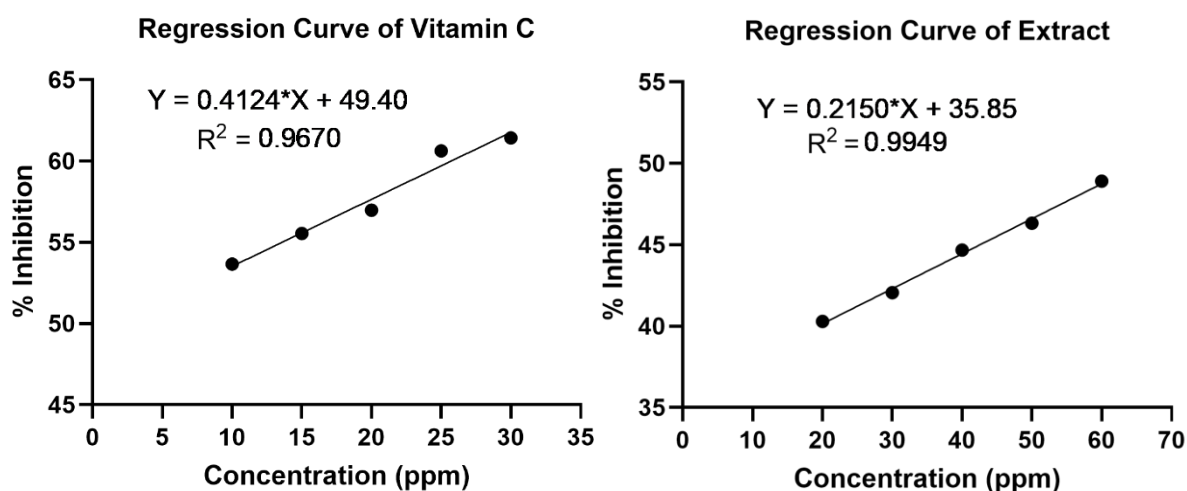


Figure 1. Linear regression curve of the ethanol extract of Chinese betel leaf and Vitamin C.

The test results show that Vitamin C (at concentrations of 10-30 ppm) provides DPPH radical inhibition ranging from 53.660% to 61.424%. The obtained  $IC_{50}$  value for Vitamin C is 1.454 ppm, confirming its potent antioxidant activity. Meanwhile, the 96% ethanol extract of Chinese betel leaf, when tested at concentrations of 20 to 60 ppm, exhibited an increase in inhibition percentage corresponding to the rising concentration. The measured inhibition range for the extract was from 40.299% to 48.913%, resulting in an  $IC_{50}$  value of 65.813 ppm. The comparison of  $IC_{50}$  values between the extract (65.813 ppm) and Vitamin C (1.454 ppm) indicates that while the Chinese betel leaf extract does possess antioxidant capacity, its potential is lower than that of the positive control. Given that the  $IC_{50}$  value is inversely proportional to activity strength (with lower values indicating stronger activity), and referring to Blois' classification [as mentioned later in your text], this ethanol extract of *Peperomia pellucida* leaves can be categorized as having strong antioxidant activity.

Table 1. Results of the antioxidant activity test of Vitamin C comparator.

Sample	Concentration (ppm)	Abs Control	Abs Sample	% Inhibition	$IC_{50}$ Value
Vit C	10	0.674	0.312	53.660	1.454
	15		0.300	55.539	
	20		0.290	56.973	
	25		0.265	60.633	
	30		0.260	61.424	

Vitamin C was used as a comparator solution (positive control) in this antioxidant activity assessment. The selection of Vitamin C was based on its status as a well-established reference antioxidant, commonly used in research. Its superior ability to reduce free radicals, along with its stability and consistency of action confirmed by previous studies [15], is the primary reason for its selection. To ensure a valid and objective comparison, Vitamin C was tested using the same DPPH protocol applied to the Chinese betel leaf extract. The data presented (Table 1) demonstrates the superior antioxidant activity of Vitamin C, as evidenced by its extremely low IC<sub>50</sub> value (1.454 ppm). This very small IC<sub>50</sub> value provides further strong evidence of the significant potential of Vitamin C in combating free radicals.

**Table 2.** Results of the antioxidant activity test of the 96% ethanol extract of Chinese betel leaf.

Sample	Concentration (ppm)	Abs Control	Abs Sample	% Inhibition	IC <sub>50</sub> Value
Extract	20	1.273	0.760	40.299	65.813
	30		0.738	42.053	
	40		0.704	44.671	
	50		0.683	46.321	
	60		0.650	48.913	

The antioxidant activity strength of the extract in this study is classified according to the system proposed by BLOIS, 1958, which uses the IC<sub>50</sub> value as a reference. According to this system, antioxidant activity is classified as: very strong (IC<sub>50</sub> < 50 ppm), strong (IC<sub>50</sub> 50–100 ppm), moderate (IC<sub>50</sub> 100–150 ppm), weak (IC<sub>50</sub> 150–200 ppm), or very weak/inactive (IC<sub>50</sub> > 200 ppm). Given that the 96% ethanol extract of Chinese betel leaf shows an IC<sub>50</sub> of 65.813 ppm, its antioxidant activity falls into the strong category.

The antioxidant activity test results of the ethanol extract of Chinese betel leaf using the DPPH method indicate that *P. pellucida* extract possesses strong antioxidant activity, with an IC<sub>50</sub> value of 65.813 ppm. Although this is higher than the positive control, Vitamin C, which has an IC<sub>50</sub> of 1.454 ppm, this result still demonstrates the potential of *P. pellucida* extract in neutralizing free radicals. When compared to other studies, such as that of Nurliansyah et al. (2024), the ethyl acetate fraction of *P. pellucida* ethanol extract showed an IC<sub>50</sub> of 6.17 ppm, and the n-hexane fraction showed an IC<sub>50</sub> of 23.84 ppm [17]. Meanwhile, Yanti et al. (2023) reported IC<sub>50</sub> values of 24.51 ppm for 70% ethanol extract and 30.50 ppm for 80% ethanol extract [14]. The differences in IC<sub>50</sub> values may be attributed to variations in extraction methods, solvent types, and the presence of active compounds, which are influenced by the geographical and environmental conditions where the plants are grown.

Although many studies have examined the antioxidant activity of *P. pellucida*, this research has several significant advantages. One of the key strengths is that the study not only evaluates antioxidant activity but also links it to potential antidiabetic properties, considering the crucial role of oxidative stress in the pathophysiology of diabetes mellitus. Additionally, the use of Vitamin C as a positive control in the DPPH assay provides a strong and valid quantitative comparison. The extract used in this study was derived from a local plant in Ternate, North Maluku, a region that has not been widely explored in similar studies, thus contributing new insights into the utilization of local biodiversity. Furthermore, the use of 96% ethanol as the primary solvent provides specific IC<sub>50</sub> data that can be compared with various solvent variations in other studies. These results also serve as a foundation for future, more specific research, such as fractionation, enzyme inhibition assays ( $\alpha$ -amylase and  $\alpha$ -glucosidase), as well as toxicity



testing and in vivo studies to support the development of *P. pellucida* as an antioxidant-based antidiabetic phytopharmaceutical candidate.

**Table 3.** Previous research data from extracts and isolates of Chinese betel leaf.

Sample	Result	References
Ethanol extract, Ethyl acetate fraction	Inhibits the activity of the enzyme $\alpha$ -glucosidase	[8]
Peperochromene A (Peperomia pellucida isolate)	Inhibits $\alpha$ -glucosidase and $\alpha$ -amylase activity	[18]
Purified ethanol extract	In vivo antidiabetic activity in alloxan-induced rats	[19]
Ethanol extract	Effectively lowers blood triglyceride levels in diabetes-induced (STZ) mice	[20]
Ethanol extract	Inhibits the activity of the enzyme $\alpha$ -amylase	[21]
8,9-dimethoxy ellagic acid (Peperomia pellucida isolate)	Significantly lowers blood glucose levels in alloxan-induced diabetic mice	[22]
The n-hexane fraction, ethyl acetate fraction, water-ethanol fraction, and ethanol extract	Has antidiabetic activity in STZ-induced mice	[23]

This finding is supported by other studies that specifically investigate the antidiabetic potential of *Peperomia pellucida* (Chinese betel leaf). Several studies have reported that both extracts and isolates from *P. pellucida* exhibit the ability to inhibit key enzymes involved in carbohydrate digestion, a crucial mechanism for controlling postprandial blood glucose elevation. For example, research by Hidayati et al. (2023) [23] found that both the ethanol extract and ethyl acetate fraction of *P. pellucida* were capable of inhibiting  $\alpha$ -glucosidase enzyme activity. Similarly, Susilawati et al. (2022) [22] reported that the isolate Peperochromene A from *P. pellucida* also showed inhibition activity against both  $\alpha$ -glucosidase and  $\alpha$ -amylase. Another study by Sciences et al. (2018) [21] also indicated the ability of *P. pellucida* leaves to inhibit  $\alpha$ -amylase enzyme activity. The inhibition of these enzymes works by slowing the breakdown of complex carbohydrates into simple sugars, thereby reducing the rate of glucose absorption into the bloodstream.

In addition to enzyme inhibition, the direct hypoglycemic effects of *P. pellucida* have also been demonstrated in vivo. Research by Sa et al. (2024) [19] showed that purified ethanol extract of *P. pellucida* had antidiabetic properties in alloxan-induced diabetic rats. Furthermore, a study by Susilawati et al. (2017) [22] successfully identified the compound 8,9-dimethoxy ellagic acid from *P. pellucida*. This isolated compound was shown to significantly reduce blood glucose levels in diabetic mice, even exhibiting effectiveness comparable to the drug glibenclamide at certain doses. The benefits of *P. pellucida* also extend beyond blood glucose control; Maryana et al. (2024) [20] reported that its ethanol extract effectively lowered blood triglycerides in diabetic rats, indicating additional potential in addressing dyslipidemia commonly associated with diabetes.

Research by Bialangi et al. (2023) also demonstrated that *P. pellucida* contains bioactive compounds such as the triterpenoid  $3\beta$ -hydroxy-9-lanosta-7,24E-dien-26-oic acid, which exhibited significant antiplasmodial activity ( $IC_{50}$  = 5.30  $\mu$ g/mL) [24]. This strengthens the hypothesis that the bioactive compound content in *P. pellucida* not only holds antioxidant potential, as demonstrated by the  $IC_{50}$  value of 65.813 ppm in this study, but could also be further developed as a multitarget therapeutic agent, including for antidiabetic and antiparasitic applications.

Overall, the findings of strong antioxidant activity in this study (IC<sub>50</sub> 65.813 ppm) further solidify the scientific evidence regarding the therapeutic potential of *P. pellucida* (Chinese betel leaf). It is strongly suspected that this antioxidant activity contributes to the plant's antidiabetic mechanism, which has previously been linked to the ability to inhibit  $\alpha$ -glucosidase and  $\alpha$ -amylase enzymes, as well as effects on reducing glucose and triglyceride levels in animal studies. The presence of bioactive compounds such as flavonoids, polyphenols, and specific components (e.g., Peperochromene A and 8,9-dimethoxy ellagic acid) is believed to be the molecular basis for these pharmacological activities, including their antioxidant properties. Therefore, the results of this study are consistent with previous findings and provide a more solid scientific foundation for the further development of ethanol extracts of *P. pellucida* leaves as a potential natural antidiabetic agent, with antioxidant activity being one of the key mechanistic contributors.

## CONCLUSION

The extract obtained from *Peperomia pellucida* (Chinese betel leaf) using ethanol as the solvent demonstrates strong antioxidant activity in neutralizing DPPH free radicals. This is evidenced by the measured IC<sub>50</sub> value of 65.813 ppm. This significant antioxidant activity underscores the extract's ability to effectively neutralize free radicals. These findings support the potential for further development of ethanol extracts from Chinese betel leaf as an antidiabetic agent, particularly considering the important role of oxidative stress in the development and complications of diabetes mellitus.

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