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Research article

Antioxidant and in vitro antidiabetic activities of *Peperomia pellucida* (L.) Kunth extract

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Abstract

Peperomia pellucida (L.) is commonly used as a herbal plant. Its effectiveness in treating inflammatory diseases, digestive disorders, and cancer in tropical and subtropical countries was introduced, especially in field of folk medicine. However, this plant species has not been studied widely in Vietnam, especially for its biological activities. This study was done to determine the antioxidant capacity of *P. pellucida* by using *in vitro* and *in vivo* methods, as well as its inhibitory ability to α-amylase enzyme activity. The total polyphenolic and flavonoid contents of *P. pellucida* extract were reported to be 359.91±0.77 mg GAE/g and 200.28±1.23 mg QE/g extract, respectively. The results showed the *in vitro* antioxidant activity of *P. pellucida* extract in four methods, including DPPH, and ABTS.+, RP and TAC, had EC_{50} values of 730.34 µg/mL, 84.33 µg/mL, 95.28 µg/mL, respectively, and Abs0.5 value of 114.73 µg/mL. Under H_2O_2-induced oxidative stress, fruit flies that were raised in the feed medium supplemented with a concentration of 1 mg/mL of *P. pellucida* extract showed their average survival time, 50% survival time, and 10% survival time at 1.6 times, 1.8 times, and 1.62 times, respectively, higher than those of the control treatment. The ability to inhibit the α-amylase activity in *P. pellucida* extract was determined with an EC_{50} value of 115.32±2.65 µg/mL compared with the commercial drug of 18.67±0, 01 µg/mL. The research results showed that *P. pellucida* is a potential species in the study of natural compounds with antioxidant and antidiabetic activities.

Keywords: Antidiabetic, Antioxidant, *Peperomia pellucida* (L.)

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INTRODUCTION

Naturally pharmaceutical sources have attracted significant attention from academia and the pharmaceutical industry due to their positive effects on human health with fewer side effects. *Peperomia pellucida* (L.) is popular and widely used thanks to its many pharmacological properties. This plant is succulent with oval leaves. It is alternate with inflorescences at the tips of spines, in the axils, and opposite the leaves. *P. pellucida* grows well in moist and porous soils with low solar radiation, especially in the rainy season (Arrigoni-Blank et al., 2002). *P. pellucida* is the herbal medicine introduced to replace synthetic drugs in healing (Florence et al., 2017). According to the traditional medicine, *P. pellucida* was applied in treating many diseases such as skin ulcers (Arrigoni-Blank et al., 2002), stomachache (Roslida and Aini, 2009), fever (Khan et al., 2008), hypotension (Kartika et al., 2016), eye diseases (Ho et al., 2022a). In addition, this plant species also have anticancer, anti-infective (Wei et al., 2011), immunostimulatory (Lee et al., 2016), antimicrobial (Zubair et al., 2015), fracture healing (Florence et al., 2017), cytotoxicity (Buhian et al., 2019), antidiabetic, anti-aging (Ho et al., 2022b) activities.

Though, in Vietnam, there have not been many studies evaluating the biological potential of this plant species. The implementation of this study was to evaluate the antioxidant activity and α-amylase inhibitory ability of *P. pellucida* extract.

MATERIALS AND METHODS

Materials

Samples of *Peperomia pellucida*, including stem and leaves, were collected in Can Tho city and identified by Dr. Nguyen Thi Kim Hue (Department of Biology, College of Natural Sciences, Can Tho University) based on morphological characteristics.

The Canton S (C.S.) strain of fruit flies used in the study was provided by the Kyoto Institute of Technology, Japan. Fruit flies were kept in a standard medium, in 1L of food consisting of agar (8 g), glucose (80 g), dry yeast (40 g), propionic acid (3 mL), cornstarch (45 g), and sodium benzoate (1 g). Food was boiled and put into experimental jars (10x4 cm), 20 mL of food for each jar. 30 fruit flies were kept in each jar and placed at a temperature of 25°C for reproduction and development (Men et al., 2019).

Methods

Extraction preparation

After being collected, the plant samples were washed, cut into small pieces, and dried naturally in shading. The dry sample was ground into a raw powder. The raw material powder (100 gram) was put into cloth bags and soaked in ethanol 96% solvent for 24 hours (ratio 1:30, weight: volume). The extract solution was filtered and collected, and fresh ethanol was added. The procedure was repeated 5 times. Finally, all extraction solutions were pooled, and ethanol was evaporated by vacuum distillation to obtain the ethanolic extract. The extracts were stored in a refrigerator at lower 4°C for further use.

Preliminary characterization of chemical composition

The biologically active compounds such as alkaloids, flavonoids, saponins, polyphenols, tannins, and steroids were preliminarily identified by using the methods described in the study of Riaz et al. (2018) and Usta et al. (2020).

Quantification of total polyphenols and flavonoids

Determination of polyphenols by Folin-Ciocalteu reagent

The content of polyphenols was determined according to the method described in the study of Dewanto et al. (2002) with modification. 250 µL of *P. pellucida* extract (1000 µg/mL), 250 µL of distilled water, and 250 µL of Folin-Ciocalteu reagent (1:4) were mixed well. 250 µL of 10% Na_2CO_3 was then added and incubated for 30 min at 40°C in a thermostatic bath. Measuring the spectral absorbance of the reaction mixture was performed at 765 nm using a 96-well spectrophotometer (Thermo Scientific, Finland). Gallic acid was a positive control that was applied to construct the standard curve equation. The content of polyphenols in *P. pellucida* extract was determined according to the standard curve equation of gallic acid.

Quantification of flavonoids with AlCl3 reagent

Flavonoids were analyzed using the method described in the study of Zhishen et al. (1999) with modification. The reaction mixture which was prepared from 200 µL of extract (500 µg/mL), 200 µL of distilled water, and 40 L of 5% $NaNO_2$, was shaken well and kept still for 5 min. Then, 40 µL 10% $AlCl_3$ was added, shaken, and continued to stay still for 6 min. When the standing time was over, 400 µL of 1M NaOH was added with distilled water to make up to 1 mL. The reaction mixture was measured its spectral absorbance at 510 nm. Quercetin was used as a positive control to construct the standard curve equation. The content of flavonoids in *P. pellucida* extract was determined based on the quercetin standard curve equation.

In vitro oxidation resistance ability

DPPH free radical neutralization method

The procedure to investigate the ability to resist oxidation of the extracts was carried out as described by Sharma and Bhat (2009) with modification. The reaction mixture consisted of 100 µL of DPPH ($6x10^{-4}$ M) and 100 µL of extract (concentration from 0 to 10 mg/mL). The reaction mixture was incubated for 60 min in the dark and at 30ºC. The reaction solution was then measured for the absorbance of DPPH at 517 nm. The positive control in the treatment was gallic acid. The formula to calculate the DPPH free radical scavenging effect is as follows:

$$E\ (\%) = (ODc - ODm)/OD_c x100.$$

Where:

E: Antioxidant efficacy (DPPH free radical neutralization ability) (%)

OD_c: The O.D. value of the negative control sample

OD_m: O.D. value of test sample

ABTS free radical neutralization method.

The method of Nenadis et al. (2004) was used to investigate $ABTS^{\bullet+}$ free radical scavenging activity. $ABTS^{\bullet+}$ solution was prepared by adding 2 mL of ABTS 7 mM solution and 2 mL of $K_2S_2O_8$ 2.45 mM solution. The mixture was incubated in the dark for 16 h, then diluted with ethanol (about 50 times), adjusted for absorbance at 734 nm with an optical density of 0.7±0.05. The $ABTS^{\bullet+}$ free radical scavenging activity of the extract was investigated by adding 990 µL $ABTS^{\bullet+}$ and 10 µL of the extract (concentration from 0 to 1 mg/mL) into the tube. The reaction mixture stayed for 6 min. The spectral absorbance at 734 nm was measured to determine the O.D. value. The higher the antioxidant capacity to neutralize $ABTS^{\bullet+}$ self-measured radicals, the lower the O.D. value measured at 734 nm. The positive control used in the treatment was gallic acid.

Reducing power (RP) method

Antioxidant activity was determined by the RP method (Oyaizu, 1986). A mixture which was prepared from 500 µL of extract at different concentrations, 500 µL of phosphate buffer (0.2M, pH = 6.6-7.2), followed by the addition of 500 µL of 1% $K_3Fe(C.N.)_6$, was kept at 50ºC for 20 min. 500 µL of 10% CCl_3COOH was then added to be taken for centrifugation at 3000 rpm for 10 min. The upper layer at the volume of 500 µL from the mixture was transferred to the Eppendorf tube and added 500 µL of distilled water and 100 µL of 0.1% $FeCl_3$. The mixture was then measured its absorbance at 700 nm. The test was repeated 3 times. Gallic acid was used to compare the results in the treatment.

Total antioxidant (TAC) method

The antioxidant activity of *P. pellucida* extract was investigated using the phosphomolybdenum method (Prieto et al., 1999). The procedure was started with the preparation of a mixture including a test sample (100 µL) at different concentrations, 1000 µL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was covered tightly and incubated at 95ºC for 90 min. The reaction solution was kept cool at room temperature, and its absorbance was measured at 695 nm. The test was repeated 3 times. Gallic acid was used to compare the result in the treatment.

In vivo antioxidant assay

In this study, H_2O_2 was used to investigate the resistance of flies to oxidative stress. Newly hatched male fruit flies within 48 h were selected and raised in standard feed conditions supplemented with extracts (concentration of 0.5 mg/mL and 1 mg/mL) for up to day 10, followed by the fasted state for 2 h; after that, the flies were placed in test vials with blotting paper H_2O_2 (concentration 10%) mixed in glucose solution 9%. The procedure was implemented 5 times (20 flies for each time). Gallic acid (0.05 mg/mL) was used as a positive control, and the control treatment used standard food. The number of survival flies was recorded every 4 hours of the survey. The values are calculated as average survival time, which is defined as the average lifespan of all surveyed individuals. The 50% survival time was calculated from the beginning of the experiment to the time when 50% of the flies were still alive. The maximum lifespan is the average survival time of the remaining 10% of the flies.

α-amylase inhibitory activity

The inhibitory activity of the extract against the α-amylase enzyme was investigated as described by Trang et al. (2012) with adjustments. The reaction mixture consisting of 50 µL of the extract, 50 µL of phosphate buffer (pH=7), and 50 µL of the α-amylase enzyme (3 U) was incubated at 37°C for 5 min. Starch (2 mg/mL) with a volume of 50 µL was added to the above mixture and continued to incubate at 37°C for 15 minutes. Then, a concentrated HCl solution with a volume of 200 µL was added to stop the reaction. Finally, 300 µL of iodine reagent solution was added to identify the residual starch after the reaction. The above mixture was measured absorbance spectrophotometrically at 660 nm.

RESULTS

Preliminary qualitative results of the chemical composition of the Peperomia pellucida extract

The qualitative analysis of the chemical composition of the extract are presented in Table 1. The results showed that *P. pellicida* extract had the presence of alkaloids, flavonoids, polyphenols, tannins, saponins, and steroids.

Table 1 The chemically qualitative analysis of *P. pellucida* extract.

Compounds	Alkaloid	Flavonoids	Polyphenols	Tannin	Saponin	Steroids
	+	+	+	+	+	+

Antioxidant capacity of P. pellucida extract

The activity of in vitro oxidation resistance

The antioxidant capacity of *Peperomia pellucida* extract was investigated by the methods of DPPH, $ABTS^{\bullet+}$, reducing power (RP), and total antioxidant capacity (TAC). The free radical scavenging efficiency determined through the EC_{50} value is presented in Table 2. The EC_{50} value results showed the ability to neutralize free radicals through DPPH, $ABTS^{\bullet+}$ and RP methods of *P. pellucida* extract was 730.3±0.7, 84.3±0.5 and 95.3±1,2 µg/mL, respectively. Abs0.5 of TAC was 114.7±1.7 µg/mL.

Table 2 *In vitro* antioxidant capacity of *P. pellucida* extract by four methods.

Sample	**EC_{50}**			**$Abs_{0.5}$**
	DPPH	**$ABTS^{\bullet+}$**	**RP**	**TAC**
P. pellucida extract (µg/mL)	730.3±0.7	84.3±0.5	95.3±1.2	114.7±1.7
Acid gallic (µg/mL)	3.6±0.3	0.4±0.0	0.7±0.0	25.4±0.2

In vivo antioxidant activity

Medicinal herbs' *in vivo* antioxidant activity was investigated through their effectiveness against oxygen stress under H_2O_2-induced conditions in flies. Under the conditions of oxidative stress caused by H_2O_2, 10% of fruit flies that were fed in a medium supplemented with gallic acid (0.05 mg/mL) and plant extracts (0.5 mg/mL and 1 mg/mL) for 10 days could prolong their tolerance to oxidative stress compared with the control (Figure 1).

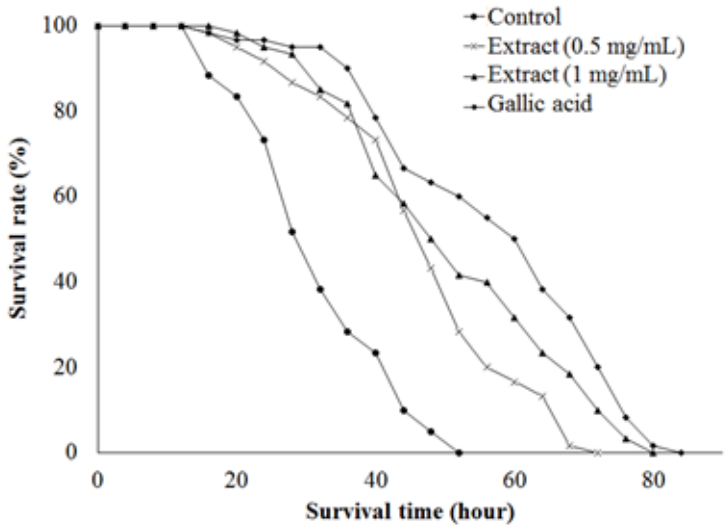

Figure 1 Effect of 10% H_2O_2 on the survival of C.S. male fruit flies which were fed in the medium supplemented with gallic acid (0.05 mg/mL) and plant extracts (0.05 mg/mL and 1 mg/mL).

Under 10% H_2O_2 condition, the *in vivo* antioxidant capacity of gallic acid, plant extracts, and the control was expressed through the average survival time, 50% survival time, and 10% survival time. The results are presented in Figure 1 and Table 3.

Table 3 *In vivo* antioxidant efficiency of gallic acid (0.05 mg/mL) and plant extracts (0.5 mg/mL and 1 mg/mL) under 10% H_2O_2 condition.

Treatment	Average survival time (hour)	50% survival time (hour)	10% survival time (hour)
Control	32.07±0.31 ^{d}	27.67±1.53 ^{d}	44.00±1.00 ^{d}
0.5 mg/mL extract	47.47±0.23 ^{c}	47.33±1.16 ^{c}	60.67±1.53 ^{c}
1 mg/mL extract	51.80±0.20 ^{b}	49.67±1.53 ^{b}	71.67±1.53 ^{b}
Gallic acid	57.80±0.20 ^{a}	60.00±1.00 ^{a}	75.33±0.58 ^{a}

Investigation of α-amylase enzyme inhibitory activity

One of the ways to treat diabetes is to reduce blood sugar spikes after eating. It is accomplished by slowing glucose absorption by inhibiting the intestinal α-amylase hydrolytic enzyme (Tundis et al., 2007). The study and use of herbal plants with blood sugar regulation activities are topics of great interest at present. This study investigated the inhibitory activity of the α-amylase enzyme from *P. pellucida* extract as shown in Table 4. The study indicated that *P. pellucida* extract could inhibit α-amylase enzyme with the EC_{50} value of 115.3±2.7 (µg/mL). However, the α-amylase inhibitory effect of *P. pellucida* extract was lower than that of acarbose (the standard compound). Acarbose is a commercial drug used for the treatment of diabetes. The results from this study demonstrated that *P. pellucida* extract might lower blood sugar by inhibiting α-amylase activity.

Table 3 The capability of inhibiting the α-amylase enzyme of *P. pellucida* extract.

Sample	EC_{50} value (µg/mL)
P. pellucida extract	115.32±2.65 ^{a}
Acarbose	18.67±0.01 ^{b}

DISCUSSION

The study of Nguyen et al., (2021) showed that the gum was found much iThe results of qualitative analysis of the chemical composition from the extract of *P. pellucida* were also similar to those reported by Sheikh et al. (2013) and Gomes et al. (2022), which demonstrated the presence of these substances in *P. pellucida*. Polyphenols and flavonoids are antioxidants that have beneficial effects on human health, curing and preventing many diseases (Tungmunnithum et al., 2018). These compounds offer many benefits in preventing and treating chronic diseases, namely cardiovascular diseases and cancer (Fraga et al., 2019). The content of total polyphenols and flavonoids in *Peperomia pellucida* extract was determined by using the linear regression equation y=0.0298-0.2692 (R^2=0.9909) based on gallic acid standard, and y=0.0047+0.0192 (R^2=0.9896) based on quercetin standard, respectively. Gallic acid is an organic acid belonging to the group of polyphenols. And quercetin is a subgroup of flavonoids. The results showed that *P. pellucida* extract had total polyphenol and flavonoid content of 359.91±0.77 mg GAE/g extract and 200.28±1.23 mg QE/g extract, respectively. In the studies of Adhitia et al. (2017), Ahmad et al. (2018), and Ng et al. (2021), polyphenols and flavonoids were determined to be present in the plant. Therefore, it can be seen that *P. pellucida* is capable of synthesizing high content of total polyphenols and flavonoids. Polyphenols and flavonoids are demonstrated to have several biological activities. These compounds can have antioxidant, anti-inflammatory, anticancer, and hepatoprotective activities (DiSilvestro, 2001; Tungmunnithum et al., 2018; Fraga et al., 2019).

The antioxidant activities of *P. pellucida* extract tested by these four methods were significantly lower than those of positive control. In particular, the total antioxidant method of *Peperomia pellucida* extract showed the best antioxidant capacity, which was 4.5 times lower than gallic acid. The results of *in vitro* antioxidant activity studies showed that *Peperomia pellucida* extract had the presence of many natural compounds, mainly polyphenolics and flavonoids. And the ability to remove free radicals from the extract was also demonstrated in the above methods. Thus, a further study investigated the *in vivo* antioxidant capacity to provide more evidence on the antioxidant capacity of the herbal extract was conducted. Besides, Table 3 showed that fruit flies that were raised for 10 days in the treatment supplemented with gallic acid (0.05 mg/mL) and extracts (0.5 mg/mL and 1 mg/mL) had a longer survival time than the control under the effect of 10% H_2O_2. The average survival time, 50% survival time, and 10% survival time of fruit flies under the treatment of 1 mg/mL extract were significantly higher than those of 0.5 mg/mL extract and control treatment. The average survival time of extract at the concentration of 1 mg/mL was 51.80±0.20 hours, 1.62 times higher than that of the control (32.07±0.31 hours). Similarly, the 50% survival time when adding *Peperomia pellucida* extract at the concentration of 1 mg/mL was 49.67±1.53 hours, 1.8 times increase compared to the control (27.67±1.53 hours). The 10% survival time in the presence of H_2O_2 was also an indicator of the antioxidant capacity of the *Peperomia pellucida* extract. In the treatment with the addition of the concentration of 1 mg/mL of the extract, the 10% survival time was 1.6 times higher than that of the control (71.67±1.53 hours compared to 44.00±1.00

hours). In addition, when adding 0.5 mg/mL *Peperomia pellucida* extract to the feed, the average survival time of flies was 47.47±0.23 hours, 1.48 times higher than that of the control (32.07±0.31 hours), 50% survival time was 47.33±1.16 hours, 1.71 times higher than the control (27.67±1.53 hours) and the 10% survival time was 60.67±1.53 hours, 1.37 times higher than the control (44.00±1.00 hours). However, the resistance to oxidative stress of *Peperomia pellucida* extract was lower than that of gallic acid. When the flies were raised in the medium supplemented with 0.05 mg/mL gallic acid, their average survival time was 57.80±0.20 hours, 1.12 times higher than those in the extract at the concentration of 1 mg/mL and 1.22 times higher than the 0.5 mg/mL concentration of the extract; the 50% survival time of gallic acid was 60.00±1.00, 1.21 times and 1.27 times higher than the concentrations of 1 mg/mL extract and 0.5 mg/mL extract, respectively; and the 10% survival time of gallic acid was 75.33±0.58, 1.05 times higher than that of the extract at the concentration of 1 mg/mL and 1.24 times higher than extract concentration of 0.5 mg/mL. This research is consistent with previous studies on the antioxidant capacity of gallic acid (Mansouri et al., 2014). The values of average survival time, 50% survival time, and 10% survival time of the extract (1 mg/mL and 0.5 mg/mL concentrations) were higher than control and lower than gallic acid, and the values have statistically significant differences ($p>0.05$). Since gallic acid is a standard commercial antioxidant with high purity, the medicinal extracts are low in purity and contain many different compounds that can inhibit each other. Ho et al. (2022b) evaluated the biological activities in *Peperomia pellucida* with the liquid-liquid partition method. The results showed that *Peperomia pellucida* had four major bioactive compounds, including dillapiole, 2,4,5-trimethoxystyrene, 9-octadecenoic acid methyl ester, and pheophorbide-a methyl ester with anti-stress and anti-aging activities. Polyphenolic, flavonoids, alkaloids, tannins, and saponins compounds have been shown to be potent antioxidants beneficial to human health, curing and preventing many diseases (Zhang et al., 2011; Tungmunnithum et al., 2018). This study demonstrated the presence of the above compounds and the maximum antioxidant capacity through experiments *in vitro* and *in vivo*. Therefore, the results of this study are consistent with previous studies.

Many studies have suggested that compounds belonging to polyphenols and flavonoids have antioxidant and antidiabetic activities. In the results of this study, the extract of *P. pellucida* has α-amylase inhibitory activity with an EC_{50} value of 115.32 µg/mL. Research by Kanedi and Mumtazah (2021) and Hidayati (2021) demonstrated that *P. pellucida* extract had anti-hyperglycemia activity as tested on rats. The results from the study of Teruna et al. (2022) also showed that the methanolic extract, n-hexane and ethyl acetate fraction slowed down the breakdown of glucose. In addition, in *P. pellucida* extract, many polyphenols, flavonoids, alkaloids, steroids, and tannins were found. Meanwhile, several studies have also shown that these compounds effectively control diabetes (Gothai et al., 2016; Fatiha et al., 2018). Flavonoids, tannins, saponins, and steroids have been reported to inhibit α-amylase activity (Aba and Asuzu, 2018).

CONCLUSIONS

Peperomia pellucida extract was evaluated for its chemical composition, quantification of polyphenols and flavonoids, antioxidant capacity *in vitro* and *in vivo*, and inhibition of the α-amylase enzyme. The results of this study confirmed that *P. pellucida* extract has strong antioxidant activity and α-amylase inhibitory activity. The content of polyphenols and flavonoids is correlated with the antioxidant capacity and inhibition of the α-amylase enzyme of *Peperomia pellucida* extract. Thus, *Peperomia pellucida* is a medicinal plant that can be used to manage and treat diabetes.

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AUTHOR CONTRIBUTIONS

Conceptualization - TTM; Methodology - TTM, LTKT and NTKA; Software - DTK; Validation - TNQ; Formal analysis - HHP, NTBN, NTAT and NTTU; Investigation - TTM, LTKT, NTAT, and HHP; Data curation - DTK and NTKA; Writing-original draft preparation - TTM, HHP, LTKT, NTKA; Writing - review and editing - DTK, NTBN, TNQ, and NTTU; Visualization - TTM and HHP; Supervision - DTK and TTM; Project administration, NTTU; Critical revisions and writing - TTM. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

We have no conflict of interest.

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