Research Article

Extraction of antioxidants from *Peperomia pellucida* L. Kunth

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Abstract

The objective of this research is to investigate the effect of two extraction techniques (maceration and reflux) and three extracting solvents (methanol, butanol and ethylacetate) on the yield and antioxidant activity of *Peperomia pellucida* L. Kunth extracts. The result showed that the methanolic extract by reflux method offered the highest extraction yield. Moreover, among the different extraction solvents, ethylacetate extract had the highest total phenolic contents. This correlated to the antioxidant activity evaluated by DPPH assay. The reducing power of extracts expressed as μmol $FeSO_4$/g extract were in the range of 0.139-1.164 μmol $FeSO_4$/g extract. Overall, higher extract yield, total phenolic contents and antioxidant activity were obtained by reflux method.

Keywords: Reflux; *Peperomia pellucida* L. Kunth; Antioxidant activity; DPPH; Medicinal plant.

1. Introduction

Peperomia pellucida L. Kunth (Pak-krasang in Thai) is also known as a plant with shiny leaves grown in many South America and Asian countries. It is an annual herb and is botanically classified in the Piperaceae family [1]. Leaves and stems of *Peperomia pellucida* have been used as food and traditional medicine for treating abdominal pain, absesses, acne, fatigue, gout, renal disorder, measles and rheumatic joint pain [2,3]. Chemical compositions were previously elucidated and indicated the presence of flavonoids, phytosterols, apiols and substituted styrenes [4]. The biological studies of crude extracts showed anti-inflammatory [5,6], antioxidant [6], bactericidal [7] and analgesic activities [5,8].

Antioxidants that are derived from plant have shown beneficial implications in preventing reactive oxygen species (ROS) and the related oxidative stress which plays an important role in several diseases such as neurodegenerative diseases [9], cancer [10], arteriosclerosis [11] and rheumatoid arthritis [12]. Consequently, a number of antioxidants from natural sources have been explored as they are safer and more beneficial compared to synthetic antioxidants [13].

One of the most frequently used techniques to recover and isolate antioxidant compounds from plant material is solvent extraction. The extraction yields and antioxidant activity of plant extract typically depend on the nature of extracting solvents/techniques due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent [14]. Different extraction procedures applied to the same plant may lead to greatly different results in efficacy of antioxidant activities of plant extract [15,16]. Therefore, this study aims to

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investigate the effective solvent and extraction method on the potent antioxidant compounds from *Peperomia pellucida* L. Kunth.

2. Materials and Methods

2.1 Chemical reagent

All chemicals were analytical grade and purchased from Sigma-aldrich. Solvents were supplied by RCI labscan, Thailand.

2.2 Preparation of plant material

Peperomia pellucida L. Kunth was collected from Amphoe Sampran in Nakornpathom province Thailand in April 2011. The sample was sun-dried and ground into powder using a blender. The powder was stored in an air tight bag at -20 °C before further experiments.

2.3 Preparation of antioxidant extracts

The plant material (20 g for each sample) was extracted by solvents including methanol, butanol and ethylacetate (400 mL each) for 24 h at room temperature (maceration) or under reflux for 45 min. Then, the filtration was applied. The solvent was removed by using rotary evaporator. The crude extracts were weighed to calculate the yield and then stored at -20 °C until used for further analyses. The yield percentage was calculated using the following formula:

$$\%\text{yield} = \frac{\text{gram of extract}}{\text{gram of dried weight}} \text{ x } 100.$$

2.4 Determination of total phenolic content

The amount of total phenolic content (TPC) was determined using the Folin–Ciocalteu method [17]. Briefly, 0.5 mL of extract was taken into a test tube. Then, 2.5 mL of Folin–Ciocalteu reagent (10x dilution) was added to this solution, and the tube was shaken thoroughly. After 5 min, 2 mL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated at 50 °C for 5 min with intermittent shaking. Absorbance was measured at 760 nm using a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg per g extract.

2.5 DPPH scavenging assay

Free radical scavenging activity of extracts was assessed using the procedure reported earlier with a slight modification [18]. Shortly, the extracts (200 μL) with nine different concentrations (0.0156, 0.0313, 0.0625, 0.125, 0.250, 0.500, 1.00, 2.00 and 4.00 mg/mL) were mixed with 800 μL of a 0.2 mM ethanolic solution of DPPH. The mixtures were incubated in the dark at room temperature for 30 min. Then, the absorbance at 517 nm was recorded. The DPPH radical scavenging activity was calculated according to the following equation, and IC_{50} was expressed as the concentration of 50% of DPPH radical scavenging activity as shown:

$$\%\text{Scavenging activity} = \left(\frac{A_C - A_S}{A_C}\right) \times 100,$$

where A_C is the absorbance of the control and A_S is the absorbance of the sample extracts.

2.6 Determination of ferric reducing antioxidant power

The total reducing capacity of extracts was determined using FRAP assay [19]. The FRAP reagent was initially prepared consisting of 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was warmed at 37 °C prior to use. Plant extracts (200 μL) were mixed with 1.8 mL of the FRAP solution and incubated for 4 min. The absorbance was then recorded at 593 nm using a spectrophotometer. The FRAP values were calculated by standard curves prepared with known concentrations of $FeSO_4$ and expressed as μmol $FeSO_4$/g extract.

2.7 Data Analysis

All analyses were carried out in triplicate, and data were reported as mean ± SD. Results were subjected to analysis of variance (ANOVA) using SPSS software (SPSS Inc., 2006). A statistically significant difference was defined at a probability value of $p<0.05$.

3. Results and Discussion

3.1 Yield of extracts

In this study, different extracting solvents, including methanol, butanol and ethylacteate used in two extraction methods i.e. reflux and maceration, were tested in order to evaluate the influence of the solvent polarity to the extract yield and the ability of extracting natural antioxidants from *Peperomia pellucida* L. Kunth. The effect of the extracting solvent/method on the yield of extract is shown in Table 1.

Table 1. Effect of extracting solvents and methods on the extract yield of *Peperomia pellucida* L. Kunth.

Solvent	% Yield	
	Maceration	**Reflux**
Methanol	$10.28\pm0.61_b^a$	$20.00\pm0.69_c^b$
Butanol	$3.04\pm0.59_a^a$	$8.63\pm0.06_b^b$
Ethylacetate	$3.06\pm0.64_a^a$	$4.07\pm0.40_a^b$

Subscript letters within the same column indicate significant ($p<0.05$) differences of means within the extracting solvents; Superscript letters within the same row indicate significant ($p<0.05$) differences of means within the extraction methods.

The yield percentage of extract was in the range of 3.04-20.00 g/100 g dry weight. The result showed that the maximum extract yield was obtained from methanol followed by butanol and ethylacetate, respectively. This is in agreement with Shon *et al*., who found that methanol and hot water were more efficient in extracting antioxidant compounds from *Phellinus baumii* [20]. Differences in the yield of extracts from plant materials in the present experiment might be ascribed to the different availability of extractable chemical compositions, extraction techniques and climate conditions [21].

For the effectiveness of extracting techniques, the results revealed that the yield of extract was better when extraction was carried out under the reflux procedure, regardless of solvent used. This suggested that hot solvent systems under the reflux condition offered higher extract yields compared to a cold solvent extraction system. Increasing the temperature of solvent reduces its viscosity and surface tension, resulting in an increase in the diffusion rate and the mass transfer rate during the extraction process. Consequently, a higher yield was obtained at high extraction temperatures [22].

3.2 Total phenolic contents of extracts

The amount of total phenolics in the extracts determined by using Folin–Ciocalteu reagent, is presented in Table 2.

Table 2. Effect of extracting solvents and methods on the TPC of extracts.

Antioxidant activity	Solvent		
	Methanol	**Butanol**	**Ethylacetate**
TPC (mgGAE/g extract)	**Maceration**		
	$25.09\pm0.53_a^a$	$42.73\pm0.81_b^a$	$93.64\pm5.64_c^a$
	Reflux		
	$35.79\pm1.10_b^b$	$109.47\pm0.98_c^b$	$121.47\pm0.32_a^b$

Subscript letters within the same row indicate significant ($p<0.05$) differences of means within the extracting solvents; Superscript letters within the same column indicate significant ($p<0.05$) differences of means within the extraction techniques.

Results of the present study demonstrated that the TPC, ranging from 25.09 to 121.47 mg GAE/g extract, varied significantly ($p<0.05$) among the extracting

solvents and extracting methods tested. Among all the solvents, ethylacetate had the highest TPC (93.64-121.47 mg GAE/g extract) while methanol showed the lowest TPC (25.09-35.79 mg GAE/g extract). This suggested that the extracts from each solvent contained a mixture of phenolic compounds at different levels according to the polarity of solvent used in the extraction process. In addition, the TPC of all extracts using the reflux technique increased, regardless of the nature of the extracting solvent used. This might be attributed to an effective extraction under reflux conditions leading to a higher release of some bound phenolics. Several investigations have shown a linear correlation between the total phenolic content and the antioxidant ability [23]. The total phenolic contents (TPC) are considered as a major group of chemicals that contribute to the antioxidant potential of medicinal plants [24].

3.3 DPPH radical scavenging activity of extracts.

The DPPH is a stable radical. It is widely employed to determine the ability to act as donor of hydrogen atoms in the transformation of the DPPH radical to its reduced form. The DPPH scavenging ability of the extracts was reported as IC_{50} expressing the amount of extract required to scavenging 50% of DPPH. In addition, less IC_{50} value generally demonstrates a high scavenging potential of a sample. The DPPH radical scavenging activities of extracts from *Peperomia pellucida* L. Kunth are shown in Tables 3.

Radical scavenging activity values (IC_{50}) of the extracts using methanol, butanol and ethylacetate as extracting solvents were 79.0-150, 87.3-240 and 74.0-110 μg/mL, respectively. As expected, extracting plant material with the reflux method offered a better IC_{50} value compared to the maceration method while ethylacetate was the best extracting solvent which gave the lowest IC_{50} value. This result was correlated to a total phenolic contents experiment. Mutee *et al.* (2010) reported that methanol extracted from *Peperomia pellucida* L. Kunth by soxhlet extraction for 3 days had an IC_{50} value of 83 μg/ml, which was higher than that recorded in our study (79 μg/mL) [6].

Table 3. Effects of extracting solvents and methods on the DPPH scavenging activity of the extract.

Antioxidant activity	Solvent		
	Methanol	**Butanol**	**Ethylacetate**
DPPH (IC_{50}, μg/mL)	**Maceration**		
	$150\pm5.60_a^a$	$240\pm24.30_b^a$	$110\pm10.60_c^a$
	Reflux		
	$79.0\pm0.50_b^b$	$87.3\pm0.11_c^b$	$74.0\pm0.52_a^b$

Subscript letters within the same row indicate significant ($p<0.05$) differences of means within the extracting solvents; Superscript letters within the same column indicate significant ($p<0.05$) differences of means within the extraction techniques.

3.4 Reducing power of extracts

The antioxidant activity of the *Peperomia pellucida* extracts was measured by FRAP assay, which is a method to determine the ability of reductants to reduce Fe^{3+} to Fe^{2+} resulting in blue coloured solution of Fe^{2+}-TPTZ complex (Fe^{2+}tripyridyltriazine). Additionally, the reducing power ability of chemical compositions in plant extracts may significantly indicate their potential antioxidant activity. The FRAP values of the extracts are summarised in Table 4.

All extracts exhibited FRAP values in the ranges of 0.651-1.164 μmol $FeSO_4$/g extract with methanol, 0.154-0.218 μmol $FeSO_4$/g extract with ethylacetate and 0.139-0.163 μmol $FeSO_4$/g extract with butanol. The FRAP values of extracts from *Peperomia pellucida* L. Kunth suggested that the best extracting solvent to achieve the greatest reducing power was methanol

and that the samples extracted by the reflux method offered the higher reducing power than those extracted by the maceration method. Moreover, the different solvents and methods revealed a significant (p<0.05) difference in the FRAP values, demonstrating that the solvents and methods influenced the ferric reducing power.

Table 4. Effects of extracting solvents/methods on the reducing power of the extract.

Antioxidant activity	Solvent		
	Methanol	Butanol	Ethylacetate
FRAP (μmolFeSO$_4$/g extract)	Maceration		
	$0.651\pm0.005_a$[a]	$0.139\pm0.004_c$[a]	$0.154\pm0.001_b$[a]
	Reflux		
	$1.164\pm0.028_c$[b]	$0.163\pm0.011_b$[b]	$0.218\pm0.001_a$[b]

Subscript letters within the same row indicate significant (p<0.05) differences of means within the extracting solvents; Superscript letters within the same column indicate significant (p<0.05) differences of means within the extraction techniques.

4. Conclusion

This study reports the effect of various extracting solvents and procedures on the antioxidant ability of *Peperomia pellucida* L. Kunth extract. It could be concluded that the antioxidant activity of *Peperomia pellucida* L. Kunth extract is drastically different among the extracts and is dependent upon the extracting solvent and extraction method used. Ethylacetate and the reflux method are recommended for increasing the efficacy of antioxidant extraction from *Peperomia pellucida* L. Kunth.

5. Acknowledgements

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6. References

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