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Effect of Gamma Irradiation on Suruhan (*Peperomia pellucida* (L.) Kunth) Herb Powder

Mubarika Sekarsari Yusuf, Intan Wulandari, Lili Amelia, Katrin, Arikadia Noviani, Rissyelly, Abdul Mun'im*

ABSTRACT

Introduction: Peperomia pellucida (L.) Kunth is known as a raw material for herbal medicine; Preservation of herbs powder by gamma irradiation is reported to be able to keep products free from contamination. **Methods:** This study aims to evaluate the effect of gamma irradiation (0; 2.5; 5; 7.5; and 10 kGy) on the ACE inhibitory activity (ACE Kit – WST test kit method), antioxidant activity (DPPH radical scavenging method), total phenolic content (colorimetric method using Folin-Ciocalteu reagent), total flavonoid content, and total flavonoid content. Antioxidant activity activity compared to others irradiated doses. Furthermore, the 5 and 7.5 kGy dose didn't cause significant change (p>0.05) on antioxidant activity, total phenolic content, and total flavonoid content. Antioxidant activity was found to correlate with the total phenolic content but not with the total flavonoid content. **Conclusion:** Based on these finding, it is concluded that gamma irradiation can be used as a preservation method for P. pellucida herb powder.

Key words: Angiotensin converting enzyme, Antioxidant activity, Total flavonoid, Total phenolic, Peperomia pellucida.

Mubarika Sekarsari Yusuf, Intan Wulandari, Lili Amelia, Katrin, Arikadia Noviani, Rissyelly, Abdul Mun'im*

Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Kampus Baru UI Depok, Jawa Barat, 16424, Indonesia

Correspondence

Abdul Mun'im

Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Kampus Baru UI Depok,

Jawa Barat, 16424, Indonesia

Phone: +62 21 727 0031

Email: munim@farmasi.ui.ac.id

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INTRODUCTION

Peperomia pellucida L. Kunth or suruhan is easily found in Indonesia and has the potential of being a herb medicine. Various studies have proven that this plant has an antioxidant and hypotensive effect.^{1,2} It's known that about 70-95% of society in developing nations use herbal medicine as their primary medication.3 Nonetheless, contamination during the processing and storage stages can shorten the shelf life of those herbal materials and even cause danger to consumers if they are contaminated by pathogenic bacteria such as Salmonella and Staphylococcus.⁴ Even though other ways of decontamination are available, gamma irradiation is preferred due it's low temperature during a process, high penetration capability, and it also doesn't produce hazardous waste.5 The aim of this study was to analyze and evaluate the effect of gamma irradiation on the chemical constituents and bioactivity of suruhan herb powder.

MATERIALS AND METHODS

Materials

Dry herb of *Peperomia pellucida* L. Kunth was collected from BALITRO (Bogor, West Java), Angiotensin-converting enzyme (ACE) Kit-WST test kit was purchased from Dojindo Laboratories, Japan, Quercetin, 2,2-diphenyl-1-picrylhydrazyl, sodium acetate and Folin-Ciocalteu reagent (Sigma-Aldrich), gallic acid, sodium carbonate and aluminium chloride, toluene, dichloromethane and methanol (Merck), and captopril (Kimia Farma Ltd, Indonesia).

Sample Preparation

Dried samples from Balitro (*Balai Penelitian Tanaman Obat dan Aromatika*), Bogor, West Java, were sorted out to separate them from pollutants, blended, then were put through a 25 mesh sieve.

Water Determination

Determination of moisture content was done with toluene distillation method.

Gamma irradiation

The sample powders (50 g) were packed with polyethylene plastic bags and irradiated using gamma with cobalt–60 as the radiation source. The dose rate was 5.79 kGy per hour with dose level of 2.5; 5; 7.5 and 10 kGy. At each dose level, three bags of 50 g of suruhan herb powder were irradiated. Irradiation was conducted at National Nuclear Energi Agency of Indonesia.

Total Plate Count (TPC) TPC was done using dilution method.

Plant extraction

Sample powder was extracted after irradiation process by reflux using 70% ethanol (1:10) for 45 minutes and repeated 4 cycles. All filtrates were combined and evaporator and was concentrated and then dried using rotary vacuum evaporator vacuum oven at 40°C.

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Free radical scavenging assay with DPPH

Various concentration of samples was prepared by dissolving the extract using methanol. About 1 mL of each concentration was put in a test tube, added 1 mL of 100 ppm DPPH solution and 2 mL of methanol pa. The mixture was vortexed and incubated for 30 minutes at 37°C. The absorbance of each concentration was measured with Uv-Vis spectrophotometer at 515 nm. Quercetin was used as the standard.

ACE inhibitory activity assay

ACE inhibitory activity assay was done following the procedure from the ACE Kit – WST test kit from Dojindo Laboratories, Japan. Captopril was used as the standard.

Determination of Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteu colorimetric methods.⁶ Different concentration of gallic acid (30-80 ppm) and sample (3000 ppm) were dissolved in methanol. About 200 μ L of each concentration was put into test tube, and 1.5 mL Folin-Ciocalteu was added. All test tubes were incubated in a dark place at room temperature for 5 minutes. Then 1.5 mL Na₂CO₃ was added to each test tube and incubated for 60 minutes in a dark place at room temperature of each concentration was measured using UV-Vis spectrophotometer at 740 nm.

Determination of Total Flavonoid Content

Determination of total flavonoid content was done using aluminium chloride colorimetric methods.^{7.8} Various concentration of quercetin (20 - 120 ppm) and sample (3000 ppm) were dissolved in methanol. About 0.5 mL of each concentration was taken in a test tube, added 1.5 mL methanol, 0.1 mL of 10 %aluminium chloride, 0.1 mL sodium acetate 1M and 2.8 mL distillate water into each test tube. Then, the test tubes were incubated at room temperature for 30 minutes. The absorbance of each concentration was measured by Uv-Vis Spectrophotometer at 434 nm.

Thin Layer Chromatography Profile

TLC profiles were performed using silica gel 60 F_{254} and dichlormethane: methanol (92:8). Ethanolic as eluent extracts were made at 10000 ppm and were spotted on the TLC plate for elusion. TLC was analyzed qualitatively under UV light of 254 nm and 366 nm, also by qualitative densitometry using TLC Scanner 3 with Camag Wincats software.

Statistical Analysis

Data was analyzed using one-way ANOVA and a significant difference was determined by the Tukey test ($\alpha = 0.05$). Correlation between antioxidant activity, total phenolic compound, and total flavonoid compound was analyzed using Spearman test.

RESULTS

Water Determination

The moisture content of suruhan herb powders obtained were 7.40% and 7.96%. This result is higher than the previous study.⁹ According to 'Peraturan Kepala BPOM Republik Indonesia' number 12; 2014 about quality requirements of traditional medicine states that moisture content of simplisia powders were less than 10%.

Total Plate Count (TPC)

Table 1 showed effect of gamma-irradiation on TPC. At dose of 2.5 kGy can reduce TPC < 10 colony/ gram.

Extraction Yields

Extraction yield of non-irradiated and irradiated simplisia herbs ranged from 21.16 – 24.86%.

Free Radical Scavenging Assay with DPPH

The results show that there is no significant difference (p>0,05) free radical scavenging activity of samples after gamma irradiation at dose 2.5 kGy, 5 kGy, and 7.5 kGy if was compared to the nonirradiated sample. Similar results on radical scavenging activity were reported for Korean herbal medicine after irradiation.¹⁴ However, there's a significant decrease (p<0,05) on radical scavenging activity of *Peperomia pellucida* after irradiation at 10 kGy (Table 2).

ACE Inhibitory Activity Assay

ACE inhibitory activity of captopril at ppm was 34.42% and all ethanol extracts were measured at 100 ppm, giving results as shown in Table 3.

Total Phenolic Content

The measurement of total phenolic content was done using colorimetric Folin-Ciocalteu method. TPC of irradiated and non-irradiated *Pepero-mia pellucida* shown in Table 4.

Total Flavonoid Content

Total flavonoid content was measured using a colorimetric method with AlCl₃ and sodium acetate as reagent. Total flavonoid content non-irradiated and after irradiated at 2.5; 5; 7.5 and 10 kGy shown in Table 5.

Table 1: Effect of irradiation on TPC of suruhan herb powder

No	Irradiation dose (kGy)	TPC (Colony/g)
1	0	296 ×106
2	2.5	< 10
3	5	< 10
4	7.5	< 10
5	10	< 10

Table 2: Antioxidant activity of non-irradiated and irradiated Peperomia pellucida herb powders

Dose (kGy)	EC _{so} ± SD
0	210.85 ± 5.98
2.5	214.60 ±8.021
5	200.83 ±5.21
7.5	199.67 ±5.76
10	279.31 ±8.48*

Each value is expressed as mean \pm standard deviation (n=3)

* significantly different (p<0.05) compare to 0 kGy

Table 3: ACE inhibitory activity of ethanol extracts of suruhan herb powder after irradiation

Irradiation dose (kGy)	Inhibitory activity (%) n=3
0	76.20
2.5	76.07
5	73.73
7.5	73.06
10	74.79

Table 4: Total phenolic content of irradiated and non-irradiated suruhan herb powder

Irradiation dose (kGy)	Phenolic content (mg GAE/g extract) ± SD
0	21.10 ± 0.11
2.5	$18.17 \pm 0.28^{*}$
5	21.24 ± 0.13
7.5	21.53 ± 0.20
10	$19.45 \pm 0.25^{*}$

Each value is expressed as mean \pm standard deviation (n=3)

* significantly different (p<0,05) compare to 0 kGy

Table 5: Total Flavonoid Content

Irradion dose (kGy)	Total flavonoid content (mg QE/g extract)
0	12.36 ± 0.40
2,5	$7.05 \pm 0.86^{*}$
5	$11.65 \pm 0,57$
7,5	13.684 ± 0.12
10	$8.586 \pm 0.47^{*}$

Each value is expressed as mean \pm standard deviation (n=3)

* significantly different (p<0,05) compare to 0 kGy

Thin Layer Chromatography (TLC) Profile

TLC profiles of all samples under 254 nm and 366 nm were similarly profile. Under 254 nm, the non-irradiated sample gave 11 peaks which were also found in the irradiated samples. Peaks were also found at R_f 0.18 and 0.67 only from the 5 kGy irradiated sample. Under 366 nm, the non-irradiated sample gave 12 peaks which were also found in the irradiated samples. Another peak was also found between Rf 0.62 and 0.64 only from the 5 kGy irradiated sample. After spraying the TLC plate with AlCl₃ solution, 10 spots demonstrated the change color into yellowish and greenish (Figure 1).

Correlation Analysis

Statistical analysis shows that there's a negative correlation between antioxidant activity with total phenolic content but there's no correlation between antioxidant activity with total flavonoid content. These results are supported by previous research which states that there's correlation between antioxidant activity with total phenolic content of *Peperomia pellucida* but have no correlation with flavonoid content.³² Total phenolic content and total flavonoid content shows a positive correlation.

DISCUSSION

Total Plate Count (TPC)

TPC of the herb powder showed a decrease of microbial growth in all irradiated samples where this result was in accordance with "Peraturan Kepala BPOM" number 12; 2014' about quality requirements of traditional medicine. Furthermore, based on 'Peraturan Menteri Kesehatan Republik Indonesia No. 701/MENKES/PER/VIII/2009' about foods irradiation, the maximum dose of dried herbs is 1 kGy to eliminate insects, and 10 kGy to eliminate pathogenic microorganisms. Gamma irradiation can kill bacteria by destroying DNA of bacteria.⁵

Extraction Yields

Ethanol was used because of its non-toxic characteristics and is permitted to be used by the Department of Health of the Republic of Indone-

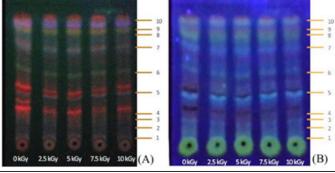


Figure 1: TLC profiles of ethanol extracts of suruhan herb powder using silica gel 60 F254 and dichloromethane: methanol (92:8) under UV light before (A) and after (B) spraying with AICI3 solution.

sia.¹⁰ A previous study reported that gamma irradiation dose of 0 to 15 kGy didn't affect extraction yield of both non-irradiated and irradiated samples.¹¹

Free Radical Scavenging Assay with DPPH

The measurement of DPPH free radical scavenging activity was based on donation of hydrogen from antioxidant to DPPH which will change the color of DPPH free radical from purple to pale yellow.¹² A significant decrease of antioxidant activity at dose 10 kGy was also reported for almond hull.¹⁴ The change of radical scavenging activity can be caused due to changes on phenolic compound because radical scavenging activity known to be correlated with phenolic acid.¹⁵

ACE Inhibitor Activity Assay

The data obtained shows there was a decrease of ACE inhibitory activity in irradiated samples. Having the least decrease was the 2.5 kGy irradiated sample. The different dose of irradiation may result in a difference of chemical constituents in the samples where a difference in the ACE inhibitory activity is allegedly caused by phytochemical constituents which are not exactly the same between samples. Every phytochemical has its own strength in ACE inhibition activity.^{16,17,18} The most significant decrease was found in the 7.5 kGy although the decrease wasn't very far from the non-irradiated sample. Such a decrease was possible due to ionization radiation effects of irradiation which can result in chemical change caused by excitation and ionization of chemical constituents.¹⁹ Gamma irradiation may also cause electron dislocation and formation of free radicals which induces breakage of chemical bonds, therefore altering chemical structures or decomposition of phytochemicals which have an ACE inhibitory activity potential.^{5,20,21}

Total Phenolic Content

The measurement of total phenolic content was done using colorimetric Folin-Ciocalteu method. A phenolic compound in alkaline will be oxidized and yields phenolate ions. The phenolate ions reduce the yellow molybdotungstophosphate heteropolyanion into blue molybdotungstophosphate, which can be measured spectrophotometrically.^{22,23} Based on statistical analysis using one-way ANOVA, it's known that there are no significant changes in total phenolic content for sample after irradiation at 5 and 7.5 kGy. The increase of phenolic content could be attributed to hydrolysis of glycosidic components by radical products from water radiolysis, leading to increasing of free phenolic compound.²⁴ Meanwhile, at dose 2.5 and 10 kGy total phenolic compound decrease significantly. The decrease of phenolic content also reported in *Mucuna pruriens* powder and almond hull after irradiation.^{14,25} The decrease of phenol content could be happened due to the interaction of radical hydroxyl from radi-

olysis with aromatic ring on phenolic compound that caused the degradation of the aromatic ring of phenolic compound.²⁷

Total Flavonoid Content

Total flavonoid content was measured using colorimetric method with AlCl₃ and sodium acetate as reagent. AlCl₃ forms acid stable complexes with keto group (C-4) and with the C-3 or C-5 hydroxyl group of flavones and flavonols. Sodium acetate detects the free hydroxyl group on C-7.^[27] Total flavonoid content after irradiated at 5 kGy and 7.5 kGy shows that there are no significant changes (p>0.05). However, total flavonoid content significantly decreases after irradiated at 2,5 kGy and 10 kGy (Table 3). In the former study, the decrease of total flavonoid content at irradiation dose 2 kGy and 10 kGy was reported for almond hull powder.¹⁵ Flavonoid is one of the polyphenol groups, thus the decrease of flavonoid content.

Thin Layer Chromatography (TLC) Profile

TLC profiles of all samples under 254 nm and 366 nm were similar. The difference of amounts peaks from the non-irradiated and irradiated samples may indicate the formation of a different substance caused by irradiation.²⁸ Another cause is the effect of gamma irradiation which produces free radicals, therefore, inducing oxidation, hydroxylation, or degradation of components in the samples.²⁹ Furthermore, decreasing or increasing of peak area of the irradiated samples is suggested as degradation result.³⁰ Colour change of the spots' fluorescence after applying AlCl₃ to greenish yellowish indicates the existence of flavonoids.³¹

CONCLUSION

Gamma irradiation of suruhan herb powder at 2.5 kGy, 5 kGy, and 7.5 kGy have no significant difference in antioxidant activity compared with control. Irradiation up to 10 kGy decreased ACE inhibitory activity of its ethanol extracts the lowest decrease was found in the 2.5 kGy irradiated sample. Total phenolic and total flavonoid content didn't change significantly compare to the control at dose 5 kGy and 7.5 kGy. There's a correlation between antioxidant activity with total phenolic content but not with total flavonoid content. TLC profiles of non-irradiated and irradiated samples gave similar results. Based on these findings, it is concluded that gamma irradiation can be used as a preservation method for suruhan herb powder.

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CONFLICT OF INTEREST

None.

ABBREVIATIONS USED

ACE: Angiotensin converting enzyme; DPPH: 2,2'-Diphenyl-1-picryl hidrazyl.

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ABOUT AUTHORS

Mubarika Sekar Sari Yusuf: Is graduated from faculty of pharmacy, Unniversitas Indonesia



Intan Wulandari: Is graduated from Faculty of Pharmacy, Universitas indonesia



Lili Amelia: Is graduated from Graduate Program of Herbal Medicine, Faculty of Pharmacy, Universitas Indonesia.



Arikadia Noviani: Is graduated from Graduate Program of Pharmacutical Scinces, Faculty of Pharmacy, Universitas Indonesia. Currently she is focusing her research in antioxidant from natural products.



Rissyelli: Is graduated in Facult of Pharmacy Universitas Indonesia. The study focused in Natural Products. Specialization course in Dyslipidemia.



Abdul Mun'im: Ph.D., Associate Professor at Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia (UI) Depok, West Java, Indonesia. He has experience in the area of Pharmacognosy and Phytochemistry, working in drugs discovery of herbal plants, extraction technology, Metabolite profiling, Structure Elucidation, and Degenerative Diseases (such as diabetes mellitus, antihypertension, and cholesterol)

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