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Original Article

Effect of Gamma Irradiation on Some Pharmacological Properties and Microbial Activities of Melinjo (*Gnetum gnemon* Linn.) Seeds

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ABSTRACT

Background: Ionizing radiation, such as gamma irradiation, serves as a useful approach to inhibit spore germination and to control pathogens in postharvest seeds. Recently, its application on phytochemical sources and its influence on antioxidant activity of various phytochemical compounds has become an interesting topic to be explored. **Objective:** The objectives of this study were to determine the effect of gamma irradiation as sterilization method on the resveratrol content and its antioxidant, HMG-CoA reductase inhibitory and dipeptidyl peptidase-4 (DPP-4) inhibitory activities of Melinjo (*Gnetum gnemon*) seeds. **Methods:** In this research, melinjo seeds were irradiated by 0.0; 2.5; 5.0; 7.5; and 10.0 kGy with gamma irradiation and then extracted with ethanol. The extracts were tested for resveratrol content with HPLC, antioxidant activities by DPPH assay, HMG-CoA inhibitory activity using HMG-CoA reductase assay kit and DPP-4 inhibitory activity using DPP-4 Inhibitor Screening Assay Kit. Gamma irradiation has effect on resveratrol content, antioxidant activity, HMG-CoA reductase inhibition and DPP-4 inhibitory activity. **Results:** From the research, the highest value of resveratrol content is 0.18±0.004 mg/g seeds powder found in 5.0 kGy gamma irradiation treatment with IC_{50} 94.64±0.236 µg/mL, while the highest HMG-CoA reductase inhibition is shown in 2.5 kGy irradiation dose. Melinjo seeds irradiated by 2.5 kGy gamma irradiation also shown a significant increase of DPP-4 inhibition activity. **Conclusion:** This study suggests that 2.5-5 kGy radiation is the effective gamma irradiation dose to improve the quality of melinjo seeds.

Key words: Antioxidant, Dipeptidyl peptidase-4, Gamma irradiation, *Gnetum gnemon,* HMG-CoA reductase, Resveratrol.

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INTRODUCTION

Melinjo (*Gnetum gnemon* Linn.) are classified as gymnosperms. Melinjo can be found in Indonesia, India, Cambodia, Vietnam, Thailand, Malaysia, Fiji, Papua New Guinea, Solomon Islands and Vanuatu.[1] Melinjo seeds contain a number of stilbenoid compounds, including trans-resveratrol (3,5,4'-trihydroxy-trans-stilbenoid), resveratrol dimers (gnetin C and gnetin L) and gnetin C glucosides (gnemonoside A, C and D).[2] Resveratrol is a phytoalexin, a secondary metabolite formed by plants. This compound is produce as a response to pathogen infection and is one of defenses against unfavorable conditions.[3] It is naturally found in various fruits including grapes, peanuts and mulberries.[4] Resveratrol showed DPPH scavenging activity that is comparable to ascorbic acid and alpha tocopherol.[5] Resveratrol and stilbene derivatives from melinjo has been known to have antioxidant,[6] antiaging and antiangiogenic activity.[7] Melinjo also has an inhibitory effect of HMG-CoA reductase with IC_{50} extract in melinjo seeds of 0.4037 µg/mL.[8] Moreover reported of 27 phenolic compounds contained in various species of *Vaccinium* and *Rubus*, in which resveratrol has the most potent activity in inhibiting dipeptidyl peptidase-4 (DPP-4) with the IC_{50} value of 0.6 ± 0.4 nM.[9]

Microbial contamination in medicinal plants may inactivate bioactive compounds due to the enzymatic activity of microorganisms. Gamma irradiation was effectively proven to control microbial contamination without adversely affecting the biologically active substances of plants.[10] Decreased bacterial growth was observed in *Terminalia chebula*, *Curcuma longa*, *Syzygium aromaticum* and *Mentha piperita* irradiated by 5.0 kGy gamma irradiation. Moreover, when irradiated by dose of 10 kGy, bacterial growths were not observed.[11] In another experiment done by Pereira *et al.* it has shown that total flavonoid content of irradiated *T. vulgaris* were increased compared to non-irradiated one.[12] The increase of total phenolic and flavonoid contents also could be related to the release of these compounds from the matrix structures, increasing extractability and the breakup rate of certain compounds to smaller compounds.[13-14]

This study was aimed to investigate the effect of ionization radiation to melinjo bioactive compounds activity such as antioxidant activity, HMG-CoA and

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DPP-4 inhibitory activity. This study also investigated the content of total phenolic and resveratrol, that is suggested to be responsible for those activities. Melinjo seeds were exposed to gamma irradiation with doses of 2.5 – 10 kGy. This study used 10 kGy as the upper limit dose in this study as it is the highest permitted dose delivered to food as written in Revised Codex General Standar for Irradiated Food.[15]

MATERIALS AND METHODS

Sample preparation

Melinjo seeds (*Gnetum gnemon* Linn.) were originally obtained from Banten, Indonesia. The sample was authenticated by Herbarium Bogoriense and the voucher specimen was deposited at Laboratory of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia. The seeds were cleaned; impurities were removed and then dried at 37°C for 48h. The skin was removed from seeds and the seeds were powdered and stored in desiccator cabinets at room temperature until further analysis.

Irradiation treatment

The seeds were irradiated with Co-60 in Gammacell 220 irradiator. The irradiation was conducted at the National Nuclear Energy Agency of Indonesia (BATAN), Pasar Jumat, Jakarta.

Determination of microbial growth

Microbial growth was determined using total plate count on Aerobic Count Plate petrifilm.[16] Yeast and mold growth was investigated using Rapid Yeast and Mold Count Plates. Serial dilutions of powder suspension were used for the growth studies. All the determinations were done in triplicates. Melinjo seeds were grounded into powders aseptically.[17] A total of 1 g of melinjo seeds powder was mixed with 0.9% NaCl solution to 10 mL to obtain 1:10 stock solution (10^{-1}). The mixture was then homogenized.

Dilutions were performed to prepare 10^{-2} to 10^{-6} suspensions. And then 1 mL of sample suspension was applied into the center of the petrifilm base layer and then closed back carefully. Petrifilm was flattened with a plastic spreader and left for 1 min to form agar. The petrifilm was incubated for 48±3 h at 35±1°C for total plate count and 60 h at 28°C for yeast and mold.[18] After the incubation period was completed, the colony forming units were observed, shown by red colour formed for total plate count and blue color for yeast and mold. The calculation of the number of colonies was adjusted to the media procedures and dilution of each suspension.

Extract preparation

Solvent extraction was performed using modified method from Wang, Liu, Chen (2013).[19] Both of the non-irradiated and irradiated powder samples were refluxed with 96% ethanol. The powder were placed in a distillation flask and mixed with 96% ethanol in a proportion of 1:6 (w/v). The mixture was left to stand for approximately 2h at room temperature. The soaked powder was extracted by reflux at 78°C for 1h. This extraction procedure was repeated three times. The extract solutions obtained were combined and filtered, then concentrated with a rotary vacuum evaporator at 65°C to obtain a crude extract of melinjo seeds (melinjo seeds extract - MSE). The solvent residue in MSE was evaporated using vacuum oven at 37°C for 48h.

Total phenolic content (TPC) determination

TPC was determined using a modified Folin-Ciocalteu colorimetric method of Bobo-Gracia *et al.*[20] The standard 20 µL gallic acid, was mixed with 100 µL Folin-Ciocalteu solution on a 96 well microplate and shaken for 60sec and then left to stand for 4 min at room temperature. Into the well added 75 µL Na_2CO_3 solution and then the mixture was shaken for 60sec and subsequently left for 2h at room temperature. Absorbance was read at 750 nm (VersaMax Microplate Reader, USA). TPC was calculated using the formula from the standard curve (12.5-200 µg/mL). The results were reported as mg gallic acid equivalents per gram seeds powder (mg GAE/g seeds powder).

Resveratrol content determination

Resveratrol (RSV) content was determined using HPLC (Shimadzu 20 LC-AT, Japan) method modified from Souto *et al.* with optimum wavelength set at 306 nm.[21] Sample was dissolved in methanol. Chromatography was equipped with 150 × 4.6 mm, 5 µm C_{18} column and UV Detector. Acetonitrile and H_2O 25:75 (v/v) adjusted to pH 3 with acetic acid were used as mobile phase. The optimum flow rate was 1.0 mL/min and the injection volume was 20 µL. RSV content was calculated using the formula from standard curve (0.2-2 µg/mL).

Antioxidant activity determination

Antioxidant activity was determined using spectrophotometer method modified from Mun'im *et al.*[22] Two milliliter samples were mixed with 1 mL DPPH solution and 1 mL ethanol. The mixture was incubated at room temperature in dark room for 30min. BHT (Sigma Aldrich, USA) was used as positive control. Absorbance was read at 516 nm using a microplate reader (VersaMax Microplate Reader, USA) and the percentage inhibition was calculated with following equation:

$$\text{Percentage of Inhibition}\,(\%) = \frac{\Delta\,\text{Control Absorbance} - \Delta\,\text{Sample Absorbance}}{\Delta\,\text{Control Absorbance}} \times 100$$

Determination of HMG-CoA reductase inhibitory activity

One hundred mg of MSE was dissolved in 100 µL DMSO. The solution was mixed with pH= 7.4 phosphate buffer to a volume of 10.0 mL to obtain a stock solution with a concentration of 10000 µg/mL. The stock solution was then diluted to 100 µg/mL working solution. HMG-CoA reductase assay kit was used to determine HMG-CoA reductase inhibitory activity, 200 µL of a working solution was mixed with 1 µL of either 100 µg/mL aqueous extract or pravastatin control. Four µL of NADPH solution, 12 µL of substrate (HMG-CoA) solution and 2 µL of HMG-CoA reductase was then added, shaken vigorously until homogeneous in a microplate reader equipped with shaker. NADPH consumption was monitored every 20 sec for 10 min by measuring absorbance at 340 nm to obtain IC_{50} for the test compound.[23] A similar process was performed with pravastatin instead the test compound to determine pravastatin IC_{50}. The IC_{50} was then determined for the test compound and for pravastatin as comparison.

Determination of DPP-4 Inhibitory Activity

The effect of the MSE on DPP-4 activity was determined fluorometrically. This measured the amount of 7-amino-4-methyl-coumarin (AMC) liberated from the DPP-4 substrate. The samples were reconstituted in double-distilled water (ddH_2O) to a final assay concentration of 25 mg/L. In a 96s-well microplate, 25 µL of test sample was mixed with 50 µL of DPP-4 (0.02 unit/mL) and 25µL ddH_2O. Subsequently 25 µL of substrate H-Gly-Pro-AMC (0.35 mM) was added and the mixture incubated at 37°C for 10 min. The enzymatic reaction was terminated by the addition of the substrate. Fluorescence of the released aminomethyl coumarin (AMC) was measured at excitation and emission wavelengths of 360 and 460 nm (GloMax Microplate Reader, USA), respectively using a microplate reader in kinetic mode for 15-30 min. Each test sample was analyzed in triplicates. Enzyme control was prepared by using ddH_2O

instead of the sample. Inhibitor control (Sitagliptin) was prepared by using inhibitor solution instead of the sample.

Statistical

Results are presented as mean ± error, in three replicates. The differences in mean was analyzed with Mann Whitney test using SPSS v.24. Statistical significance was interpreted as $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 lists microbial growth, total resveratrol content, TPC and antioxidant activity of melinjo seeds at various irradiation doses. It shows that increasing doses of irradiation influence resveratrol content, TPC and antioxidant activity with the significance level $p \leq 0.05$.

Microbial Growth

Melinjo seeds are prone to microbial contamination during cultivation, harvesting, storage, distribution and sales. Such contamination may have a significant impact on the quality and shelf life of the product.[10] In food stuffs, molds often responsible for decay and production of mycotoxins.[24] Aerobic plate count was chosen because it is easy to prepare and use compared to conventional agar plate. Aerobic count plate includes almost all aerobic bacteria and facultative anaerobes in the sample. The growth rate of bacteria was determined by counting the number of red colonies after incubation 37±1°C for 48±3 h, in accordance with the guidelines on aerobic plate count. The result of this total plate count determination in all test samples with six concentrations and the blank did not show bacterial growth after the incubation period. This means that samples of non-irradiated melinjo seeds are sterile from bacteria (Table 1).

Dose of gamma irradiation 2.5 kGy effectively kills mold and yeast in melinjo seeds. In non-irradiated melinjo seeds, there were 400 cfu/g mold growth (Table 1). Gamma radiation produces ionization which causes substrate chemical change that inactivates microorganisms. The energy produced by ionizing radiation directly affecting microbial DNA molecules, causing damage to the cells of the mold or bacteria.[25] Gamma irradiation also produces indirect effect, in which irradiation energy produced causes the hydrolysis of water molecules in the substrate or irradiated material that produce free radicals and ions that attack the microorganisms DNA.[26-28] The minimum inhibitory dose (MID) dose of gamma irradiation for 10^8 *Aspergillus ochraceus* spore is approximately 2.5 kGy.[29] These data are consistent with results obtained from our experiment, which shows that 400 cfu/g of mold in melinjo seeds can be removed by exposure 2.5 kGy gamma irradiation.

Total Phenolic Content (TPC)

Table 1 presented the effect of gamma irradiation of MSE on TPC. Determination of TPC was performed using Folin-Ciocalteu as reagent and gallic acid as standard.[30] The reaction is the transfer of electrons with the basic medium of the phenolic compound to the phosphomolibdat or phosphotungstate acid complex shown by the formation of blue complexes in the solution.[31] The formation of blue color is proportional to the amount of phenol compounds contained. The addition of Na_2CO_3 is to provide basic condition for the reaction process because the reaction is condition.[32]

The total phenolic content of the non-irradiated MSE was smaller compared to the irradiated sample. The highest total phenolic content was observed in the 5 kGy dose. It is known that irradiation which may increase total phenol content, varies from starting dose as low as 2.5 kGy;[33] 3 kGy;[34] 4 kGy.[35-36] Irradiation may cause the degradation of tannins,[37] break the chemical bond of polyphenol compounds and release low soluble phenol compounds with low molecular weight.[38] Irradiation produces radical compounds that may affect chemical components such as phenolic compounds.[39] However, at doses of 7.5 kGy, the total phenol content is decreased. However, at 10 kGy irradiation doses, the phenolic contents were observed to be increasing again (higher than the 7.5 kGy sample). This phenomenon is similar with resveratrol content. Based on these results, it is suggested that the optimum dose of irradiation is 5 kGy because it gives the highest total phenol content.

Table 1: Effect of gamma irradiation on microbial growth, resveratrol content, total phenolic content and antioxidant activity of melinjo seeds.

Irradiation dose (kGy)	Bacteria (cfu/g)	Mold-yeast (cfu/g)	Resveratrol content (mg/g seed powder)	Total phenolic content (Gaye/g seed powder)	IC_{50} of antioxidant activity (µg/mL)
0.0	0	400	0.093±0.006	11.11±0.059	223.50±6.298
2.5	0	0	0.162±0.005	13.52±0.209	112.21±0.513
5.0	0	0	0.185±0.004	15.01±0.240	94.64±0.236
7.5	0	0	0.097±0.002	11.29±0.136	122.67±1.916
10.0	0	0	0.133±0.009	14.15±0.229	113.69±1.414

Value are expressed as mean± error measurement (n=3). Means on the same line are significantly different ($p \leq 0.05$).

Resveratrol Content

The effect of gamma irradiation on resveratrol content of MSE is presented in (Table 1). Resveratrol content on MSE non-irradiated (0 kGy) is smaller than the irradiation samples. Irradiation may increase the concentration of phytochemical compounds of samples or materials.[37] The highest level was obtained on MSE with 5 kGy irradiation dose 0.185±0.004 mg/g of seeds powder. The results indicate that 5 kGy is optimum dose for irradiation of seeds melinjo.

Antioxidant Activity

The effect of gamma irradiation on antioxidant activity of MSE is presented by IC_{50} value (Table 1). Both non-irradiated and various irradiated dose have significant difference ($p \leq 0.05$). IC_{50} value on non-irradiated MSE (0 kGy) is 233.55±6.293 µg/mL. These results show that MSE has weak antioxidant activity. In this research, irradiation showed to be able to increase antioxidant activity, indicated by the decrease of IC_{50} value compared to resveratrol standard, IC_{50}: 8.5 µg/mL.[40]

Samples with irradiated doses of 5 kGy has the highest antioxidant activity of 94.64±0.236 µg/mL which showed as strong antioxidants. Gamma irradiation did not influence antioxidant activity of MSE. Irradiation can increase antioxidant activity based on dose, exposure time, solvent for extraction and sample used.[40-41] Increasing antioxidant activity after irradiation caused by fragmentation of the polysaccharide chain, forming low molecular weight compounds and releasing hydroxyl groups thereby reducing hydrogen intermolecular bond.[38] In addition, irradiation causes molecular degradation into phenol compounds and activation of the enzyme phenylalanine ammonia-lyase.[42] Decreasing antioxidant activity after a dose of 5 kGy related to the irradiation effect on a particular membrane or compound. Based on the results obtained showed that the optimum dose of irradiation is 5 kGy. Meanwhile the doses above 5 kGy may cause degradation of the bioactive compounds that has antioxidant activity. However the rate of degradations in 10 kGy is lower than 5.0 kGy. This results are consistent with TPC and resveratrol content. Further study need to be done to investigate the relation between TPC content and various doses between 5 – 10 kGy. As in this research we only had done two different doses hence no clear relation was observed.

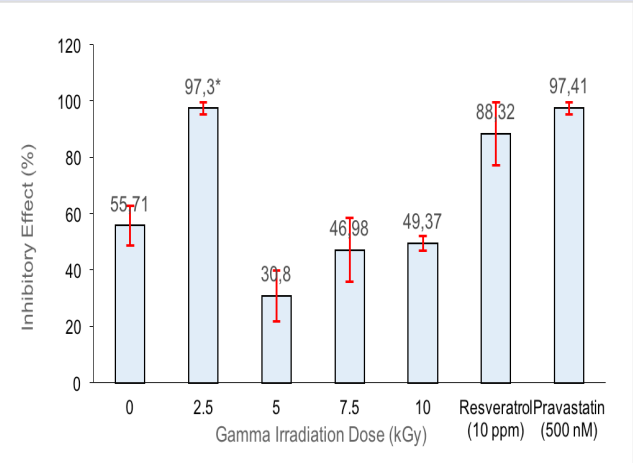

Figure 1: HMG-CoA reductase inhibitory activity of MSE. Each sample is expressed as mean of triplicate experiments. Gamma irradiation at 2.5 kGy dose shows significant difference compared to 0.0, 5.0, 7.5, and 10.0 kGy. Pravastatin (500 nM) was used as positive control. Asterisk (*) shows the data with significant differences.

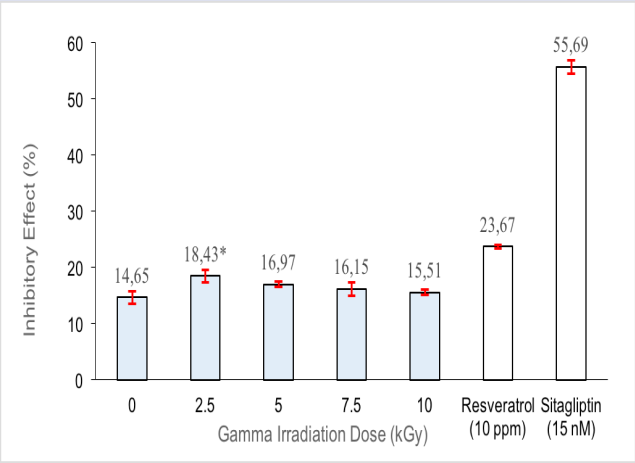

Figure 2: Inhibitory activity of MSE on DPP-4. Each value is expressed as mean in triplicate experiments. Gamma irradiation treatment 2.5 kGy to 10.0 kGy showed no significant diffrence compared to nonirradiated control (0.0 kGy). Sitagliptin (15 nM) was used as positive control. Asterisk (*) shows the data with significant differences.

HMG-CoA Reductase Inhibitory Activity

Based on the results of inhibition percentage test from five samples (Figure 1), 2.5 kGy irradiated melinjo seeds has shown the highest inhibition percentage, 97.3%, relatively close to pravastatin standard, 97.41%. MSE with a 2.5 kGy irradiation dose is potentially nutritious substance as anticholesterol through inhibition of HMG-CoA reductase. While for other irradiation doses, the percentage of inhibition for the extracts of 0.0, 5.0, 7.5 and 10.0 kGy were 55.71%, 30.80%, 46.98% and 49.37%, respectively. In statistical analysis, the difference between inhibition percentage of 2.5 kGy is significant ($p<0.05$). While for dose irradiation 5, 7.5 and 10 kGy indicate the percentage of inhibition that is not significant each other. The percentage of inhibition of MSE with irradiation dose of 2.5 kGy is significantly higher because certain compounds capable of inhibiting HMG-CoA reductase in MSE. 5 kGy of irradiated melinjo seeds in this experiment shows lower activity compared to non-irradiated sample. It might be inferred that the concentration of active compounds, such as resveratrol, are lower in the higher dose of irradiation.

Gamma rays are categorized as ionizing radiation and can interact with atoms or molecules to produce free radicals. This radical compound may damage or modify important compounds in plant cells and is known to affect morphological, anatomical, biochemical and physiological changes, depending on their dose.[39] In another study related to irradiation effect on jujube fruit to its bioactive compound, it was found that at 2.5 kGy irradiation dose caused the increasing of total monomer anthocyanin and total phenolic content significantly. However, the content of these bioactive compounds decreased after irradiation at a dose of 5.0 kGy. Gamma irradiation at doses greater than 2.5 kGy in melinjo seeds showed a significant reduction of total phenolic content. This is may caused by degradation by free radicals as suggested from previous research.[33]

Research conducted by Cantos, García-Viguera, de Pascual-Teresa, Tomás-Barberán (2000) related to the effects of ultraviolet radiation B and C on resveratrol showed that the resveratrol content of the skin of grapes increased with the treatment of ultraviolet irradiation.[43] Ultraviolet light is known to induce resveratrol biosynthesis faster. The irradiation conditions used were 10 times lower than those was conducted by Roggero and Garcia-Parrilla (1995) which resulted in damage to resveratrol.[44]

In this research, it was also known that temperature and storage time affected the amount of resveratrol content in both irradiated and non-irradiated samples. The content of resveratrol underwent an erratic change as temperature and storage duration also increased variously.

Based on the results of the above research, it is known that the difference of HMG-CoA reductase inhibition in this study is caused by the influence of gamma irradiation and may supported by several other factors such as temperature and storage time of MSE.

DPP-4 Inhibitory Activity

Irradiation showed no significant changes on inhibitory activity of DPP-4 (Figure 2). The increase of inhibitory activity of DPP-4 at 2.5 kGy could be due to increase of phenylalanine ammonia-lyase (PAL) activity, Oufedjikh, Mahrouz, Amiot, Lacroix, (2000) reported that gamma irradiation (0.3 kGy) caused a significant increase ($P \leq 0.05$) of PAL activity on the *Citrus clementine* Hort fruit peel, compared to controls.[45] In another study, Benoît, D'Aprano, Lacroix, (2000) also reported that gamma irradiation 0.5 kGy to 2.5 kGy increase PAL and total phenolic activity in *Agaricus bisporus*.[46] As explained by Halls, Yu, (2008) about the role of PAL in the resveratrol biosynthesis pathway, PAL catalyzes the elimination of phenylalanine into cinnamic which is a precursor for the formation of a number phenolic compounds including resveratrol.[47]

Gamma irradiation effect on inhibitory activity of DPP-4 experiment provides a simple overview that 2.5 kGy gamma irradiation caused an increase in DPP-4 inhibitory activity. But at higher doses, the activity actually decreases. These data are similar with the research by Ayed, Yu, Lacroix in 2000 which reported that low-dose gamma irradiation (<2.0 kGy) may not alter anthocyanin compound in wine.[48] However at higher doses, anthocyanin shows to be decreasing. In another study by Najafabadi, Sahari, Barzegar, Esfahani at 2017, it was reported that 2.5 kGy gamma irradiation significantly increased total monomeric anthocyanin and phenolic total, but at dose of 5.0 kGy, there was a significant decrease in both components.[33] Anthocyanin is a flavonoid compound synthesized through the phenylpropanoid pathway, anthocyanin, resveratrol or other phenolic compounds also products of PAL.[49] An increased percentage of DPP-4 inhibition at a dose of 2.5 kGy but decrease in higher gamma irradiation doses was suggested to be related

to the effects of gamma irradiation on PAL. In addition, Momesso *et al.* (2009) reported that gamma irradiation at high doses (20.0 kGy) causing compound degradation and antioxidant activity loss of resveratrol.[50]

Similar to the findings from previous experiment, this experiment results showed that irradiation at 2.5, 5.0, 7.5 and 10.0 kGy increases melinjo seeds DPP-4 inhibition activity compared to non-irradiated sample. However, if irradiation doses given were too high (>5 kGy), the inhibitory activity was lower compared to 2.5 kGy as dose with the highest activity in this research.

CONCLUSION

Evaluation of ionizing radiation effect on melinjo seeds in this experiment shows that gamma irradiation increases resveratrol content, antioxidant activity, inhibition of HMG-CoA reductase and DPP-4 activity. There is no bacteria growth observed in both non-irradiated and irradiated melinjo seeds, while dose as low as 2.5 kGy has shown to effectively inhibit molds and yeasts growth. This study suggests that 2.5-5 kGy radiation is the effective gamma irradiation dose to improve the quality of melinjo seeds.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

MSE: Melinjo Seeds Extract; **DPPH:** 1,1-diphenyl-2-picryl-hydrazyl; **BHT:** Butylated hydroxytoluene; **HMG-CoA:** 3-Hydroxy-3-methyl-glutaryl–coenzyme A; **NADPH:** Nicotinamide adenine dinucleotide phosphate; **DMSO:** Dimethyl sulfoxide; **DPP-4:** Dipeptidyl peptidase-4; **AMC:** 7-Amino-4-methyl-coumarin; **PAL:** Phenylalanine ammonia-lyase; **TPC:** Total Phenolic Content; **MID:** Minimum inhibitory dose; **HPLC:** High performance liquid chromatography; RSV: Resveratrol; **PVS:** Pravastatin; **STG:** Sitagliptin.

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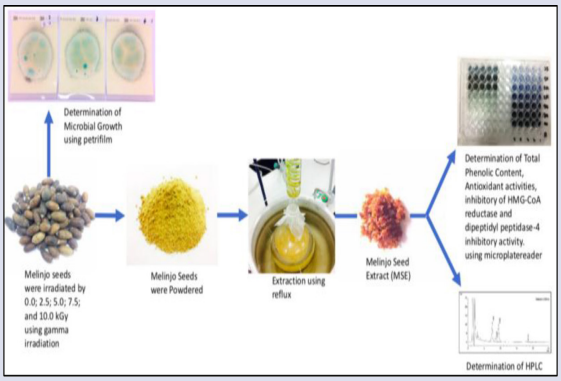
GRAPHICAL ABSTRACT



SUMMARY

- The present study provides the evaluation of effectiveness gamma irradiation as microbial decontamination method of Melinjo (*Gnetum gnemon* Linn.) Seeds and its effect on resveratrol content, total phenolic content, antioxidant activity, HMG-CoA reductase and Dipeptidyl peptidase-4 inhibitory activity of Melinjo (*Gnetum gnemon* Linn.) Seeds.

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