



Research article

Effect of gamma irradiation on the caffeoylquinic acid derivatives content, antioxidant activity, and microbial contamination of *Pluchea indica* leavesErnawati^{a,b}, Herman Suryadi^c, Abdul Mun'im^{d,*}^a Graduate Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, 16424, West Java, Indonesia^b Directorate of Traditional Medicines, Health Supplements and Cosmetics Registration, Indonesian Food and Drug Authority, Jakarta, 10560, DKI Jakarta, Indonesia^c Department of Microbiology and Pharmaceutical Biotechnology, Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, 16424, West Java, Indonesia^d Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, 16424, West Java, Indonesia

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ABSTRACT

Pluchea indica (L.) Less. leaf has a long history of being used as a food and in traditional medicines. Although gamma irradiation is an effective decontamination method, it must be performed appropriately to preserve the bioactive constituents and biological activities of the plant. This study investigated the influence of gamma irradiation on the caffeoylquinic acid derivatives content, antioxidant capacity, and microbial burden of *P. indica* leaf. Dried *P. indica* leaf powder was exposed to gamma rays from cobalt-60 at the absorbed doses of 2.5, 5.0, 7.5, and 10 kGy. After a maceration of *P. indica* leaf with 70% ethanol, the content of six caffeoylquinic acid derivatives (CQAs) in the extract was assayed using high-performance liquid chromatography. The antioxidant capacity of the ethanolic extract was also determined using the DPPH, ABTS, and ferric reducing antioxidant power (FRAP) methods. The total aerobic bacteria and total yeast and mold counts were investigated using the Petrifilm method at 0 and 3 months after irradiation. Doses of 5–10 kGy significantly increased the CQA level ($P < 0.05$). The antioxidant activity was enhanced significantly at 2.5–10 kGy ($P < 0.05$). Doses of 2.5–10 kGy also effectively reduced the microbial load ($P < 0.05$). Among the irradiation doses, 10 kGy showed the best results. Thus, gamma irradiation at 10 kGy is useful in increasing CQA content and antioxidant capacity as well as reducing the microbial load of *P. indica* leaf.

1. Introduction

Caffeoylquinic acid derivatives (CQAs) are natural phenolic compounds that belong to the chlorogenic acid family. Recently, plants rich in CQAs have gained attention since CQAs possess beneficial biological properties, such as acting as antioxidants, alpha-glucosidase inhibitors, and HIV-1 integrase inhibitors as well as having anti-inflammatory and anti-cancer effects (Arsiningtyas et al., 2014; Naveed et al., 2018; Shukri et al., 2011; Srisook et al., 2012; Wianowska and Gil, 2019). For example, the leaves of *Pluchea indica* (L.) Less. (*beluntas*), a member of the Asteraceae family, contain CQAs as their main component (Arsiningtyas et al., 2014; Kongkiatpaiboon et al., 2018; Shukri et al., 2011). *P. indica* leaves also contain other compounds, such as flavonoids (quercetin, kaempferol, myricetin, luteolin, and apigenin), caffeic acid, anthocyanins (Andarwulan et al., 2010; Suriyaphan, 2014), and volatile oil [(10S,

11S)-Himachala-3-(12)-4-diene] (Widyawati et al., 2013). *P. indica* leaves are commonly consumed as food and traditional medicine in Southeast Asia, China, India, Australia, and America. For example, young leaves and shoots are usually made into vegetable salads, soups, or side dishes. Herbal tea made from *P. indica* leaf is also commercially available in Indonesia and Thailand. Traditionally, *P. indica* leaves have been used to treat diarrhea, fever, lumbago, leucorrhoea, gangrenous ulcer, body odor, and applied as an antidiabetic, nerve tonic, or diuretic (Andarwulan et al., 2010; Suriyaphan, 2014; Widyawati et al., 2014).

Gamma irradiation is gaining significant interest as one of the most promising and widely used decontamination methods for foods and herbal materials. Low doses at less than 1 kGy of gamma irradiation have been applied to inactivate parasites such as protozoa and helminths in meat products, fresh fruit, and vegetables. Meanwhile, medium doses at 1–10 kGy have been used to reduce or eliminate non-viral

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microorganisms and extend the product shelf life of fresh, frozen, and dried foods and spices. Additionally, high doses at 10–60 kGy have been utilized for microorganisms reduction or sterilization of foods, such as dry ingredients and foods for hospital patients and astronauts (J. Farkas, 2006; Farkas and Mohácsi-Farkas, 2011; IAEA, 2015; Munir and Federighi, 2020; Pereira et al., 2018, 2017; WHO, 1981). Moreover, gamma irradiation at 15 kGy is effective for decontaminating polycyclic aromatic hydrocarbons in wheat kernels and pea seeds (Khalil & Al-Bachir, 2015, 2017). The use of gamma irradiation has been approved in Indonesia and more than 60 countries globally (Munir and Federighi, 2020). Gamma irradiation is preferred over other decontamination methods because it effectively eliminates microorganisms without leaving chemical residues, making it safe and environmentally friendly. Gamma irradiation is also efficient, fast, and convenient as it can be applied at room temperature. Additionally, it can be applied to packaged foods to prevent recontamination after irradiation (Farkas, 1998; Pereira et al., 2018, 2017).

Gamma irradiation can trigger water radiolysis that generates reactive oxygen species (ROS), such as the free radicals $\cdot\text{OH}$, $\text{H}\cdot$, $\text{O}_2^{\cdot-}$, and $\text{HO}_2\cdot$. The free radicals, especially $\cdot\text{OH}$, primarily cause damage in DNA and other macromolecules, leading to microorganism death. However, these free radicals can modulate the plant's ROS and antioxidant levels, damage or modify bioactive components, and cause the accumulation of phenolic compounds (Gudkov et al., 2019; Jan et al., 2012; Reisz et al., 2014). Many researchers have studied the effect of gamma irradiation on phenolic compounds and antioxidant activity of foods and medicinal plants. Some studies have found that gamma irradiation has positive effects, while others observed insignificant or even negative effects. For example, gamma irradiation at 0.5 and 1 kGy in minimally processed baby carrots reduces the total phenol content by 10% and 20%, respectively (Hirashima et al., 2013). In addition, Ito et al. (2016) showed that a dose at 1 kGy preserved the phenolic compound content and antioxidant activity of apple pomace flour more effectively than 2 kGy. In contrast, Hussain et al. (2016) revealed that doses of 0.25–1.5 kGy increased the phenolic compounds and antioxidant activity of fenugreek and spinach. Additionally, doses of 1 and 10 kGy preserve most of the 11 phenolic compounds identified in *Aloysia citrodora* Palau leaf (Pereira et al., 2017) and increase the levels of phenolic compounds, particularly lithospermic acid in *Melissa officinalis* L. and 5-O-caffeoylquinic acid in *Melittis melissophyllum* L. (Pereira et al., 2018). Khawory et al. (2020) also demonstrated that gamma irradiation of 3–13 kGy elevated the total phenolic content and antioxidant capacity of *Gnetum gnemon*, *Euodia malayana*, and *Khaya senegalensis* leaves.

The influence of gamma irradiation on the phenolic compounds and antioxidant activity will depend on the irradiation dose. It is crucial to investigate the specificity of the product and the sensitivity of each phytochemical compound to irradiation before determining the appropriate irradiation dose for a particular herb (Ito et al., 2016). Six CQAs viz. 5-O-caffeoylquinic acid (5-CQA), 4-O-caffeoylquinic acid (4-CQA), 3-O-caffeoylquinic acid (3-CQA), 4,5-O-dicaffeoylquinic acid (4,5-diCQA), 3,5-O-dicaffeoylquinic acid (3,5-diCQA), and 3,4-O-dicaffeoylquinic acid (3,4-diCQA) have been reported to have strong antioxidant activities because they possessed the oxidizable ortho-diphenol group, which could act as a radical scavenger and prevent radical chain reactions (Xu et al., 2012). There is a strong correlation that these six CQAs are responsible for the antioxidant activity of *P. indica* leaf extract (Vongsak et al., 2018). On the other hand, CQAs are characterized by their chemical instability. MonoCQAs and diCQAs undergo rapid degradation under the influence of both temperature and light radiation together. Increasing temperature and light exposure induces the isomerization, transesterification, or degradation of CQAs to other compounds (Wianowska and Gil, 2019; Xue et al., 2016). However, the effect of gamma irradiation on the CQA levels and antioxidant capacity of *P. indica* leaf has not been investigated. In this study, we examine the influence of gamma irradiation on the caffeoylquinic acid derivatives

content, antioxidant activity, and microbial contamination of *P. indica* leaf.

2. Materials and methods

2.1. Chemicals and reagents

Unless otherwise stated, all chemicals and reagents were of analytical grade and were purchased from Merck KGaA (Darmstadt, Germany). Methanol for liquid chromatography, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (purity >90%) (Merck KGaA, Darmstadt, Germany); the standards 4,5-diCQA, 3,5-diCQA, 3,4-diCQA, 5-CQA, 4-CQA, 3-CQA (Chengdu Biopurify Phytochemicals Ltd., Chengdu, China), all with a purity of >98%, except for 3,5-diCQA, which had a purity of >95%; ascorbic acid (Indonesian FDA, Jakarta, Indonesia); potassium persulfate, 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ferrous sulfate heptahydrate, and 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Plant material

P. indica leaves were collected from Pati district, a lowland area (altitude between 0 and 100 m above sea level) with a tropical climate in Central Java, Indonesia, at the beginning of November 2020 (early wet season). *P. indica* leaves (Asteraceae) were authenticated by the Research Center For Plant Conservation and Botanic Gardens, Indonesian Institute of Science. Fresh *P. indica* leaves (juvenile and mature) were sorted to remove unwanted materials or impurities, washed with clean water, and dried in direct sunlight for 5 days. Dried leaves were crushed, filtered through a 60-mesh sieve, and packaged in polyethylene ziplock pouches. Then, they were stored in an airtight glass bottle at room temperature (15–30 °C) with ambient relative humidity and protected from direct sunlight until being used.

2.3. Determination of water content and water activity

The water content of the dried *P. indica* leaf powder was determined using the azeotropic method (toluene distillation) (Ministry of Health Republic of Indonesia, 2017), and the experiment was performed in triplicates. Meanwhile, the water activity was measured using an AW meter, and the experiment was performed in duplicates.

2.4. Gamma irradiation treatment

Dried leaf powder of *P. indica* was exposed to gamma rays from ^{60}Co using a *Gammacell 220* irradiator with a dose rate of 4.1 kGy/h, at various absorbed doses (2.5, 5, 7.5, and 10.0 kGy). Irradiation was performed by certified personnel at the National Nuclear Energy Agency of Indonesia (BATAN).

2.5. Preparation extract of *P. indica* leaves

Dried *P. indica* leaf from each irradiation dose was macerated separately using 70% ethanol (1:10 w/v) for 24 h with occasional shaking during the first 6 h following the procedure stated in the Indonesian Herbal Pharmacopoeia II (Ministry of Health Republic of Indonesia, 2017). After 24 h, the macerate was separated by filter paper (Whatman No. 1). The marc was reextracted twice, but the amount of solvent was reduced to half of the amount of solvent at the first maceration (1:5 w/v). All filtrates were pooled and evaporated using a rotary vacuum evaporator (Buchi, Switzerland) at 50 °C. The crude extracts were weighed and stored in an airtight container at 0 °C until further analysis. The extraction of each irradiation dose was performed in triplicates.

2.6. Caffeoylquinic acid assay via high-performance liquid chromatography

The effect of gamma irradiation on the bioactive content was evaluated by examining six CQAs (4,5-diCQA, 3,5-diCQA, 3,4-diCQA, 5-CQA, 4-CQA, and 3-CQA) following the method developed by Kongkiatpairoon et al. (2018) with some modifications as described below.

2.6.1. Chromatographic condition

LC-20AD (Shimadzu, Kyoto, Japan), equipped with a DGU-20A5 degasser, CBM-20A, SIL 20AC HT autosampler, CTO-20A column thermostat, and SPD-M20A photodiode array detector, was used. The six CQAs were separated using a Zorbax Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm) with a C18 guard column (4.6 × 12.5 mm, 5 μm) (Agilent Technologies, Santa Clara, CA, USA). The mobile phase comprised 0.5% acetic acid in water (A) and methanol (B). Gradient elution was programmed as follows: 10% B to 50% B for 40 min, 50% B to 100% B for 2 min, 100% B for 8 min, 100% B to 10% B for 2 min, and 10% B for 8 min. The injection volume, flow rate, and column temperature were 5 μL, 1.0 mL/min, and 40 °C, respectively. The CQAs were detected using a PDA detector at λ326 nm.

2.6.2. Standard and sample preparation

Each standard was carefully weighed and dissolved in methanol to obtain a 1.0 mg/mL stock solution and was then filtered using a 0.2 μm nylon syringe filter (Agilent Technologies, Santa Clara, CA, USA). The mixed standard solutions of 160, 80, 40, 20, 10, 5, and 2.5 μg/mL were made from stock solutions diluted with methanol. *P. indica* leaf extract was carefully weighed, dissolved in methanol, and sonicated for 30 min until completely dissolved. Before analysis, the sample was filtered with a 0.2 μm nylon syringe filter.

2.6.3. Method validation

Method validation was conducted according to ICH guidelines (ICH, 1995/2006) (European Medicines Agency, 2006). Linearity, precision, accuracy, limit of quantitation (LOQ), and limit of detection (LOD) were evaluated. Linearity was examined using mixed standard solutions at concentrations from 160–2.5 μg/mL. The measurement for each concentration was done three times. The peak area was plotted versus concentration to obtain calibration curves for each standard. The 20 μg/mL mixed standard solution was analyzed six times within a day to evaluate intraday precision and was examined for three consecutive days to determine interday precision. The accuracy analysis was performed using the standard addition method. Three concentrations of mixed standard solution were added to *P. indica* leaf extract 0 and 5 kGy, at approximately 50%, 100%, and 150% of the determined content in the sample. Three independent spiked samples were prepared for each percentage level. Precision was evaluated by %RSD, and recovery was calculated using Eq. (1).

$$\text{Recovery (\%)} = \frac{\text{Amount found} - \text{Original amount}}{\text{Amount spiked}} \times 100 \quad (1)$$

The linearity of the analyte added into the spiked sample was assessed at five concentrations. Different concentrations of mixed CQA standard, at approximately 50%, 75%, 100%, 125%, and 150% of the determined content in the sample, were added to *P. indica* leaf extract 0 and 5 kGy. Two independent spiked samples were prepared for each percentage level. Standard solutions with known low concentrations were measured for their signal-to-noise ratios (S/N), in which the LOD and LOQ were defined as S/N = 3 and S/N = 10, respectively.

2.7. Determination of the DPPH radical scavenging activity

The DPPH radical scavenging experiment was performed following the method described by Vongsak et al. (2018) with slight adjustments.

Methanol was used to dissolve DPPH, *P. indica* leaf extract, and ascorbic acid (as a positive control). In a 96-well microplate, 100 μL of DPPH solution (100 μg/mL) was added to 100 μL of *P. indica* leaf extract (10–50 μg/mL) or ascorbic acid (6–14 μg/mL). As the control, 100 μL of methanol was added to 100 μL of DPPH solution. The microplate was shaken and incubated at 37 °C for 20 min in the dark. The absorbance was measured at λ517 nm using a microplate reader (VersaMax, San Jose, CA, USA). The experiment was performed in triplicate, and the percent of radical inhibition was calculated according to Eq. (2).

$$\text{Percent inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100 \quad (2)$$

The relationship between the test solution concentration and percent inhibition was tested using linear regression. From the regression equation, the IC₅₀ was calculated as the concentration to obtain 50% radical inhibition.

2.8. Determination of antioxidant activity by the ABTS^{•+} method

The analysis was performed using the method described by Vongsak et al. (2018) with slight modification. Radical ABTS^{•+} was generated by mixing equal parts (1:1) of 4.9 mM potassium persulfate and 14 mM ABTS aqueous solution. The mixture was incubated for 16–20 h in the dark at room temperature, and then 2 mL of this mixture was diluted with 52 mL ethanol to obtain the ABTS working solution. In a 96-well microplate, 100 μL of ABTS working solution was added to 100 μL of ethanol to obtain a control absorbance of 0.70 ± 0.02. For sample testing, 100 μL of ABTS working solution was added to 100 μL of *P. indica* leaf extract (5–60 μg/mL) or ascorbic acid (4–12 μg/mL). After incubation for 6 min at room temperature in the dark, the absorbance was measured at λ734 nm. The analysis was performed three times. The calculation of percent inhibition and IC₅₀ was performed according to Eq. (2).

2.9. Determination of ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was conducted following the method described by Vongsak et al. (2018) with minor adjustments. The FRAP working solution for the sample or positive control was prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃ (10:1:1). In the FRAP working solution for the calibration curve, FeCl₃ was replaced with double-distilled water. Methanol was used to dissolve the *P. indica* extract and ascorbic acid. In a 96-well microplate, 150 μL of FRAP working solution was mixed with 50 μL of *P. indica* extract (40 μg/mL) or ascorbic acid (12 μg/mL) and was incubated at 37 °C for 8 min. As a reference, ferrous sulfate heptahydrate (60–220 μM) was analyzed to make a calibration curve. Determination of absorbance was conducted at λ593 nm using a microplate reader. The test was done in triplicate. The FRAP values are presented as grams of FeSO₄ equivalent per 100 g sample.

2.10. Microbe quantification

Irradiated and nonirradiated (0 kGy) dried *P. indica* leaf powder samples were tested for the total aerobic plate count (TAPC) and total yeast and mold (TYM) immediately and 3 months after irradiation using the Petrifilm method according to AOAC Official Method 990.12 (1990) and AOAC Official Method 997.02 (1997), respectively (Curiale et al., 1990; Knight et al., 1997). Briefly, 10 g of dried *P. indica* leaf powder was aseptically weighed and placed into a sterile stomacher bag with a filter, after which 90 mL sterile 0.9% NaCl solution was added, and the solution was mixed until homogenous to obtain a suspension with a 10⁻¹ dilution; 1 mL of suspension was transferred into 9 mL of sterile saline solution, resulting in a dilution of 10⁻²; 1 mL of the diluted sample suspension

(10^{-2}) was mixed with 9 mL sterile saline solution to make a subsequent dilution of 10^{-3} .

Test samples (1.0 mL each) were inoculated onto the center of a 3M™ Petrifilm™ Aerobic Count Plate and 3M™ Petrifilm™ Yeast and Mold Count Plate. The inoculum was distributed using a 3M spreader, and the film was left for at least 1 min until the gel solidified. As a negative control, 1.0 mL of sterile saline solution was inoculated on the film. The inoculation was performed in triplicate for each level of dilution. Bacterial colonies were counted after 24 ± 3 h of incubation at $35 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$. The yeast and mold colonies were counted after 5 d of incubation at $28 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$.

2.11. Statistical analysis

The results of repeated measurements are presented as mean \pm standard deviation. Mean differences between the irradiated and control samples were analyzed using one-way analysis of variance (ANOVA) followed by post hoc test by Tukey's HSD to assess significant differences between each group. For the TAPC, TYM, and FRAP assays, the Games–Howell post hoc test was applied. Differences were considered significant at $P < 0.05$. Statistical analysis was performed with Jeffrey's Amazing Statistics Program (JASP) Version 0.14.1 (JASP Team, 2020).

3. Results and discussion

The water content and water activity of *P. indica* leaf powder were determined before irradiation. The mean of water content was 7.2%, which met traditional medicine requirements in Indonesia (Indonesian FDA, 2019). However, water activity is a more critical parameter than water content in affecting microbial growth. Water activity (a_w) is defined as the ratio of the water vapor pressure in the material (p) and the vapor pressure in pure water (p_0) at the same temperature (Müller and Heindl, 2007). Water activity describes the availability of free water that can be utilized for microbial growth. The minimum a_w required for growth varies depending on the type of microorganism, but in general, there is no microbial proliferation at an a_w below 0.61 (Barbosa-Cánovas et al., 2020). The mean of water activity in the *P. indica* specimen was 0.448, a level below which microorganisms can reproduce.

P. indica leaf powder was treated with gamma irradiation at 10, 7.5, 5, and 2.5 kGy. An absorbed dose of 10 kGy was set as the highest dose in this study based on the FAO/IAEA/WHO Joint Expert Committee on the Wholesomeness of Food Irradiation (JECFI), food irradiation up to 10 kGy did not pose a toxicological hazard; hence, no toxicological testing was required. Food irradiation up to 10 kGy also introduced no special nutritional or microbiological problems (WHO, 1981). As stated in the Manual of Good Practice in Food Irradiation, medium-dose (1–10 kGy) applications are recommended for reducing microbiological contamination in spices and dried food ingredients (IAEA, 2015). In addition, 10 kGy is the maximum absorbed dose permitted by the Indonesian FDA to reduce pathogenic microbes in dried vegetables, seasonings, dry herbs, and herbal teas (Indonesian FDA, 2018). Both the irradiated and control (0 kGy) samples showed no difference in physical appearance.

3.1. Effect of gamma irradiation on the extraction yield

Caffeoylquinic acids are phenolic compounds that are relatively polar and are soluble in ethanol or ethanol-water mixtures (Wianowska and Gil, 2019). Hence, 70% ethanol was chosen as the solvent. Additionally, 70% ethanol is commonly used to extract *P. indica* leaf in commercial products. The mean of extraction yields ranged from 25.68% to 28.15% (w/w) and showed no significant difference between the irradiated and control samples ($P > 0.05$).

3.2. Effect of gamma irradiation on CQAs

In 1976, the IUPAC published a new numbering system of CQAs that reversed the order of atomic numbering in the quinic acid ring, where chlorogenic acid (3-CQA) is now designated 5-CQA (Naveed et al., 2018). In this study, we use the old numbering system for CQAs; chlorogenic acid (CAS No. 327-97-9) is defined as 3-CQA, and neochlorogenic acid (CAS No. 906-33-2) is 5-CQA.

The applied HPLC method can separate the mixture of six CQA compounds with a resolution of >2 (Figure 1). Based on their retention times, the polarity order of the compounds was 5-CQA $>$ 3-CQA $>$ 4-CQA $>$ 3,4-diCQA $>$ 3,5-diCQA $>$ 4,5-diCQA. This result is similar to that of a previous study by Kongkiatpaiboon et al. (2018) and CQA testing using reversed-phase HPLC (Wianowska and Gil, 2019). Tables 1, 2, and 3 present the validation results. The applied method showed good linearity, within 2.5–160 $\mu\text{g/mL}$, with a coefficient of determination (r^2) of ≥ 0.999 . The RSD values for intraday and interday precision were 0.96%–1.35% and 1.54%–2.09%, respectively, indicating good repeatability. The LOD values (0.04–0.5 $\mu\text{g/mL}$) and LOQ values (0.12–0.17 $\mu\text{g/mL}$) of the six CQAs were low, demonstrating high sensitivity. Similar results were also reported by Kongkiatpaiboon et al. (2018), where the LOD and LOQ values for these six CQAs compounds were 0.03 and 0.1 $\mu\text{g/mL}$, respectively.

In the spiked sample at 0 kGy, the average percent recovery ranged from 96.95% to 102.93% with an RSD of 0.03%–3.44%, whereas in the spiked sample at 5 kGy, the accuracy was in the range of 91.67%–104.65% with an RSD of 0.22%–4.58%. A linearity evaluation of analyte in the spiked sample at 0 and 5 kGy were also conducted. The results showed good linearity (Table 3). Thus, the method was suitable for determining the six CQAs in irradiated and nonirradiated *P. indica* leaf extracts.

P. indica leaf extracts from all irradiation doses had the same analyte profile, where the content of 3,5-diCQA was the highest among the six CQAs (Figure 2). Generally, as shown in Table 4, the CQA content tended to increase in the irradiated samples. Compared to the control (0 kGy), there was no significant reduction in the monoCQA levels in the irradiated groups. The level of 5-CQA was significantly increased at 2.5, 5, and 10 kGy. Meanwhile, at 7.5 kGy, the amount of 5-CQA was similar to that at 0 kGy. Moreover, the level of 4-CQA was significantly elevated only at the dose of 10 kGy. Irradiation dose did not affect 3-CQA significantly. However, 3,4-diCQA increased at doses of 5 and 10 kGy. At doses of 5, 7.5, and 10 kGy, 4,5-diCQA increased considerably, but the level of 3,5-diCQA was similar to that at 0 kGy. Interestingly, in the 2.5 kGy sample, the content of diCQAs (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) decreased significantly, but the level of 5-CQA significantly increased. According to Xue et al. (2016), diCQAs are less stable than monoCQAs and can degrade into their cis-isomer or methylated forms. Additionally, 3,5-diCQA can transform into 5-CQA (Wianowska and Gil, 2019; Xue et al., 2016). Energy from gamma irradiation might cause the degradation of diCQAs more readily than that of monoCQAs, such as 3,5-diCQA transforming into 5-CQA, but this assumption should be investigated further.

Interestingly, as Figure 2 and Table 5 show, four unknown analytes (A, B, C, and D) were detected in *P. indica* leaf extract with a large peak area, especially for peaks C and D. These analytes were also affected by gamma irradiation. Analyte A tended to decline, with a significant decrease at 2.5 and 10 kGy. The other analytes also decreased at 2.5 kGy, but analyte C increased significantly at 5 and 10 kGy, whereas analyte D significantly increased at 5, 7.5, and 10 kGy. There was no significant difference in analyte B at 5, 7.5, and 10 kGy compared to that at 0 kGy.

Based on the UV spectrum (Figure 3), analyte A has maximum absorption at λ_{255} and 350 nm, indicating that compound A might belong to the flavonoid group. It is known that flavonoids have two main band

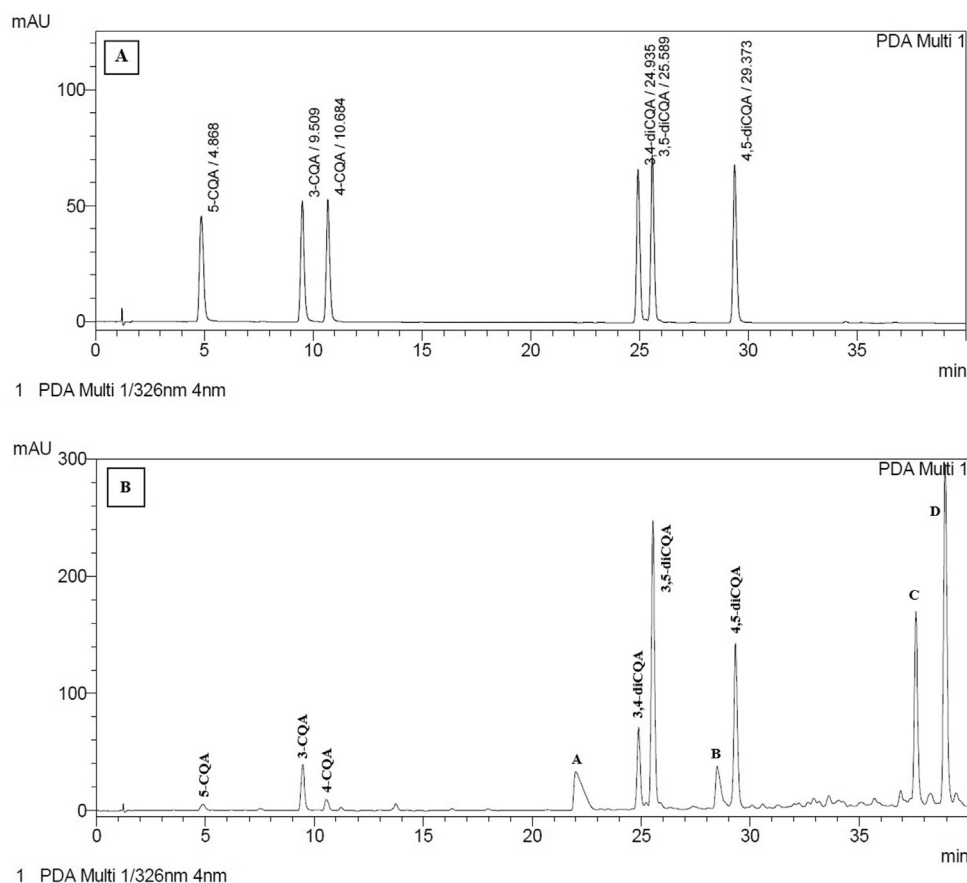


Figure 1. HPLC chromatograms of the six caffeoylquinic acid derivatives in the mixed standard solution (A) and *P. indica* leaf extract 0 kGy (B). 5-CQA: 5-O-caffeoylquinic acid; 3-CQA: 3-O-caffeoylquinic acid; 4-CQA: 4-O-caffeoylquinic acid; 3,4-diCQA: 3,4-O-dicafeoylquinic acid; 3,5-diCQA: 3,5-O-dicafeoylquinic acid; 4,5-diCQA: 4,5-O-dicafeoylquinic acid; A, B, C, and D: unknown analytes.

Table 1. Method validation results.

Parameter	Results					
	5-CQA	3-CQA	4-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
Regression equation*	$y = 14739x + 3710.6$	$y = 14881x + 291.5$	$y = 14721x + 390.7$	$y = 17322x - 2678.5$	$y = 18889x - 3666.9$	$y = 19189x - 2389.8$
Coefficient of determination (r^2)	0.9993	0.9996	0.9996	0.9996	0.9996	0.9996
Linear range ($\mu\text{g/mL}$)	2.5–160	2.5–160	2.5–160	2.5–160	2.5–160	2.5–160
Intraday precision (%RSD)	1.35	1.09	1.03	1.11	1.01	0.96
Interday precision (%RSD)	1.71	1.57	1.54	1.75	2.09	1.70
LOQ ($\mu\text{g/mL}$)	0.17	0.16	0.15	0.13	0.12	0.14
LOD ($\mu\text{g/mL}$)	0.05	0.05	0.05	0.04	0.04	0.04

* x is analyte concentration, and y is peak area.

absorptions at 240–285 nm (band I) and 300–560 nm (band II) (Sammani et al., 2021). Analytes B, C, and D might belong to the CQA compounds because their UV spectra are similar (Figure 3). Based on the retention time, compound B is probably dicafeoylquinic acid as its polarity is similar to 4,5-diCQA. Meanwhile, analytes C and D have a retention time greater than that of 4,5-diCQA, indicating that they are less polar than dicafeoylquinic acid compounds; hence, they might be tricaffeoylquinic acid compounds. However, these assumptions need further investigation.

Increasing CQA content in irradiated samples might be due to the effect of gamma irradiation on enhancing phenylalanine ammonia-lyase (PAL) activity (Jan et al., 2012; Oufedjikh et al., 2000). Biosynthesis of CQAs is through the phenylpropanoid pathway. PAL plays a critical role in the first step of the pathway, which catalyzes the conversion of phenylalanine into cinnamic acid (Clifford et al., 2017). There is a

positive correlation between irradiation dose and PAL activity in *Agaricus bisporus* mushrooms (Benoit, D'Aprano and Lacroix, 2000), *Prunus persica* fruit (Hussain et al., 2010), and *Rosmarinus officinalis* calli (El-Beltagi et al., 2011). Additionally, gamma irradiation could break the chemical bonds of polyphenols, thereby releasing soluble phenols of low molecular weight (Jan et al., 2012).

Similar to our results, several studies have reported the positive effect of gamma irradiation on phytochemical content. Gamma irradiation doses up to 12.5 kGy increase the content of phenolic compounds, flavonoids, saponins, and tannins in *Ziziphus mauritiana* leaves, likely due to the release of active compounds from the more complex structures degraded by gamma rays (Khattak and Rahman, 2016). In addition, a dose of 10 kGy elevates the phenolic content of persimmon leaf extract and mulberry leaf extract (Cho et al., 2016, 2017), enhances the

Table 2. Recovery and precision of analytes in the spiked sample extracts at 0 and 5 kGy.

Spiked Level	Analyte	% Recovery (mean \pm SD, n = 3)		Precision (%RSD)	
		0 kGy	5 kGy	0 kGy	5 kGy
1 (50%)	5-CQA	98.80 \pm 0.03	91.67 \pm 0.61	0.03	0.67
	3-CQA	100.81 \pm 0.77	97.13 \pm 1.83	0.77	1.88
	4-CQA	101.50 \pm 0.72	101.20 \pm 4.63	0.71	4.58
	3,4-diCQA	99.67 \pm 0.59	102.81 \pm 3.76	0.59	3.66
	3,5-diCQA	99.84 \pm 2.49	101.30 \pm 2.23	2.50	2.20
	4,5-diCQA	101.55 \pm 2.75	104.65 \pm 3.73	2.71	3.57
2 (100%)	5-CQA	100.04 \pm 1.42	100.06 \pm 0.30	1.42	0.30
	3-CQA	99.39 \pm 3.26	101.01 \pm 0.51	3.28	0.51
	4-CQA	96.95 \pm 1.57	102.55 \pm 1.71	1.62	1.67
	3,4-diCQA	100.04 \pm 2.37	100.01 \pm 0.33	2.37	0.33
	3,5-diCQA	102.93 \pm 3.54	96.13 \pm 4.06	3.44	4.22
	4,5-diCQA	102.31 \pm 0.60	100.08 \pm 2.05	0.59	2.05
3 (150%)	5-CQA	98.83 \pm 1.13	101.04 \pm 0.24	1.13	0.23
	3-CQA	100.02 \pm 0.89	100.01 \pm 0.42	0.89	0.42
	4-CQA	100.04 \pm 0.17	100.07 \pm 1.04	0.17	1.04
	3,4-diCQA	99.08 \pm 0.65	99.23 \pm 0.35	0.66	0.35
	3,5-diCQA	100.04 \pm 0.65	100.05 \pm 2.36	0.65	2.36
	4,5-diCQA	100.10 \pm 1.76	100.07 \pm 0.22	1.76	0.22

Table 3. Linearity of analytes in the spiked sample extract at 0 and 5 kGy.

Analyte	0 kGy		5 kGy	
	Regression equation	r ²	Regression equation	r ²
5-CQA	y = 14603x + 693.55	0.9992	y = 15308x - 6082.8	0.9981
3-CQA	y = 13923x + 5948.7	0.9980	y = 14305x + 9388.8	0.9984
4-CQA	y = 13256x - 7490.29	0.9948	y = 13419x + 4631	0.9923
3,4-diCQA	y = 16229x + 7818.5	0.9992	y = 16280x + 54640	0.9943
3,5-diCQA	y = 18978x - 22046	0.9980	y = 20127x + 17811	0.9964
4,5-diCQA	y = 18816x - 1695.9	0.9977	y = 20089x + 17125	0.9989

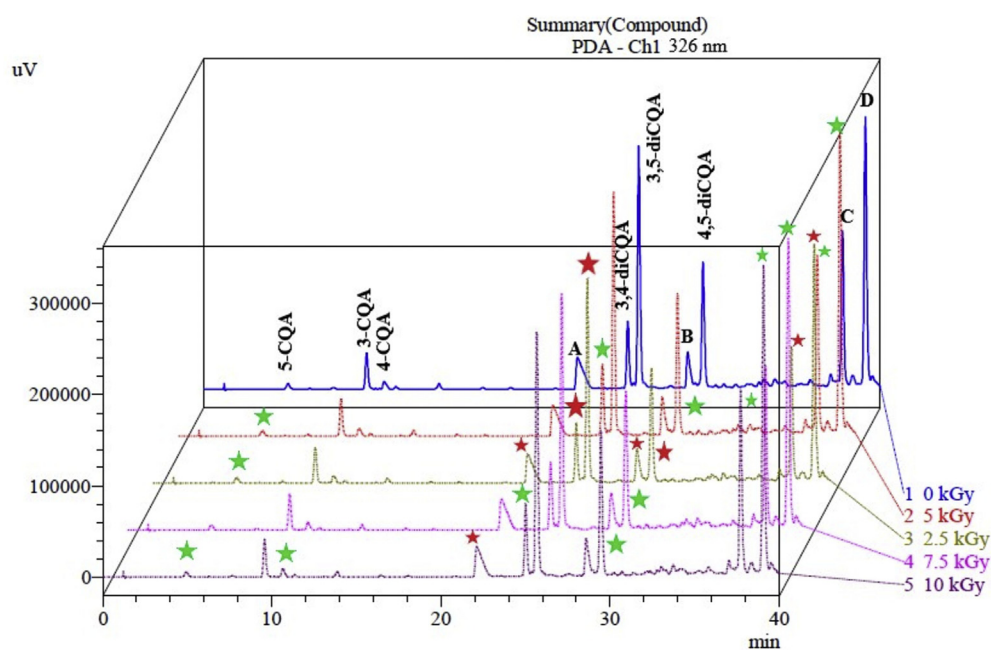
**Figure 2.** HPLC chromatograms of *P. indica* leaf extract under various irradiation doses. (1) 0 kGy, (2) 5 kGy, (3) 2.5 kGy, (4) 7.5 kGy, and (5) 10 kGy, detected at λ 326 nm. ★: Significantly increased compared to the control (0 kGy). ★: Significantly decreased compared to the control (0 kGy).

Table 4. The effect of gamma irradiation on the content of six CQAs in *P. indica* leaf extract.

Irradiation dose (kGy)	Content of CQAs (% w/w)*					
	5-CQA	3-CQA	4-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
0.0	0.119 ± 0.002 ^c	0.695 ± 0.010 ^{ab}	0.187 ± 0.004 ^{bc}	0.988 ± 0.014 ^b	3.301 ± 0.074 ^a	1.915 ± 0.047 ^c
2.5	0.129 ± 0.002 ^a	0.685 ± 0.014 ^b	0.179 ± 0.006 ^c	0.899 ± 0.021 ^c	2.854 ± 0.080 ^b	1.764 ± 0.028 ^d
5.0	0.125 ± 0.002 ^b	0.712 ± 0.008 ^a	0.195 ± 0.005 ^{ab}	1.051 ± 0.005 ^a	3.339 ± 0.039 ^a	2.121 ± 0.024 ^{ab}
7.5	0.121 ± 0.003 ^c	0.698 ± 0.010 ^{ab}	0.186 ± 0.007 ^{bc}	0.998 ± 0.016 ^b	3.278 ± 0.086 ^a	2.088 ± 0.034 ^b
10.0	0.126 ± 0.001 ^{ab}	0.710 ± 0.007 ^a	0.199 ± 0.005 ^a	1.063 ± 0.017 ^a	3.308 ± 0.077 ^a	2.170 ± 0.030 ^a
Average	0.124	0.700	0.189	1.000	3.216	2.012
Minimum	0.119	0.685	0.179	0.899	2.854	1.764
Maximum	0.129	0.712	0.199	1.063	3.339	2.170

Different letters for the same analyte measurement represent significant differences between the doses at $P < 0.05$.

* Data are expressed as mean ± SD (n = 6).

Table 5. The effect of gamma irradiation on the unknown analytes A, B, C, and D in *P. indica* leaf extract.

Irradiation dose (kGy)	Peak area of unknown analyte (mean ± SD, n = 6)*			
	A	B	C	D
0	1,006,024.25 ± 29,091.11 ^a	521,920.97 ± 7,488.44 ^{ab}	1,742,703.69 ± 48,493.64 ^b	3,155,179.23 ± 83,238.40 ^c
2.5	928,137.68 ± 20,303.64 ^b	469,733.97 ± 17,887.54 ^c	1,507,977.00 ± 31,537.15 ^c	2,821,768.86 ± 55,818.73 ^c
5	991,361.80 ± 5,747.24 ^a	541,081.15 ± 10,213.35 ^a	1,962,910.85 ± 29,704.31 ^a	3,497,096.64 ± 58,243.07 ^{ab}
7.5	1,003,351.14 ± 13,965.42 ^a	518,562.92 ± 7,393.64 ^b	1,805,959.41 ± 44,145.88 ^b	3,404,148.55 ± 89,589.55 ^b
10	918,001.24 ± 21,143.02 ^b	539,347.38 ± 17,961.26 ^{ab}	2,001,966.36 ± 37,109.57 ^a	3,526,717.23 ± 57,569.15 ^a

*Different letters within the same column represent significant differences between the doses at $P < 0.05$.

isoflavone content of Serbian soybean (Popović et al., 2013), and increases the total phenolic and flavonoid content of mugwort extract (Hwang et al., 2021). The flavonoid and phenolic contents were also augmented at 5 kGy in Sakouti and Bondoky dry date fruits, likely due to the enhancement of PAL, tannin degradation, release of compounds from its glycosidic form, and degradation of polymeric phenolic compounds into low molecular weight phenolic compounds (El-Beltagi et al., 2019). The enhancement of phenolic contents after exposure to gamma irradiation is also observed in black soybean extract (Krishnan et al., 2018), *Prunus persica* fruit (Hussain et al., 2010), Malaysian honey (Hussein et al., 2011), *Gnetum gnemon* seed (Syahdi et al., 2019), and *Arthrospira (Spirulina) platensis* (Shabana et al., 2017).

On the other hand, some studies have demonstrated the negative effects of gamma irradiation. For example, a dose of 5 kGy reduces the total phenol and flavonoids contents of *Cuscuta chinensis* L. extract by 12% and 18%, respectively (Alijaniha et al., 2021). Meanwhile, a dose of 10 kGy significantly decreases the total phenolic and flavonoid contents of Sakouti and Bondoky dry date fruits (El-Beltagi et al., 2019). Lastly, doses of 2 and 4 kGy reduce the tannin and total phenolic content of soybean grains (de Toledo et al., 2007).

In addition, other studies have reported that gamma irradiation up to 10 kGy does not affect the phenolic compounds in *Thymus vulgaris* and *Mentha x piperita* leaves (Pereira et al., 2016). Gamma irradiation also causes no significant changes in the total polyphenols, flavonoids, and flavonols of *Polygoni Multiflori Radix* (Chiang et al., 2011). Moreover, Khattak and Simpson (2010) demonstrated that doses up to 15 kGy exerted no significant effect on the phenolic content of *Glycyrrhiza glabra* root; however, doses of 20 and 25 kGy increased the phenolic content. Furthermore, Ghadi et al. (2017) reported that doses of 1, 2, and 10 kGy did not affect the level of chlorogenic acid of *Cichorium intybus* roots; however, doses of 4, 6, and 8 kGy significantly elevated the level of chlorogenic acid and total phenolic contents, and a dose of 10 kGy decreased the total phenolic content. In *Nigella sativa* seeds, Khattak et al. (2008) reported that doses up to 16 kGy did not change the phenolic content in methanol extract. However, the phenolic content was increased in acetone extract and decreased in water extract, indicating

that the solvent used also played an important role in assessing the effect of gamma irradiation on phenolic content.

Variations in the effect of gamma irradiation on the phytochemical content might be due to multiple factors, such as irradiation dose, plant species, sample type (solid or dry), extraction solvent and method, characteristics and structures of phytochemical compounds, environmental and geographical conditions, and temperature (Khattak and Rahman, 2016).

The CQA amounts in this study differed from the CQA concentrations reported by Kongkiatpaiboon et al. (2018) and Vongsak et al. (2018), where the content 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA were within the range of those found in *P. indica* from various locations in Thailand, but 5-CQA, 3-CQA, and 4-CQA were lower. The discrepancy in the levels of these active compounds is understandable because many factors influence them. Differences in methods of drying and extraction, type of solvent, level of maturity, environmental conditions, and harvest time could affect the chemical composition of plants (Chewchida and Vongsak, 2019; Jeng et al., 2015; Kongkiatpaiboon et al., 2018; Vongsak et al., 2018; Wianowska and Gil, 2019; Widyawati et al., 2014). Vongsak et al. (2018) showed that young *P. indica* leaves have a higher CQA content than mature leaves. According to Kongkiatpaiboon et al. (2018), the CQA content in *P. indica* from locations with high rainfall and humidity was higher than that from dry plateaus.

3.3. Effect of gamma irradiation on the antioxidant capacity

Assessment of the influence of gamma rays on the antioxidant activity of *P. indica* leaf extract was performed using the DPPH, ABTS, and FRAP methods. These methods were chosen because they are suitable for hydrophilic antioxidants, and they are simpler, faster, more reproducible, and cheaper than other testing models. DPPH and ABTS are based on the reduction of DPPH• and ABTS•+ free radicals by antioxidant compounds that donate hydrogen atoms. In the DPPH assay, the presence of antioxidants causes a color change from purple to yellow, which can be measured at $\lambda 517$ nm. The bluish-green ABTS•+ radical decolorizes when reduced to ABTS, which can be determined at $\lambda 734$ nm.

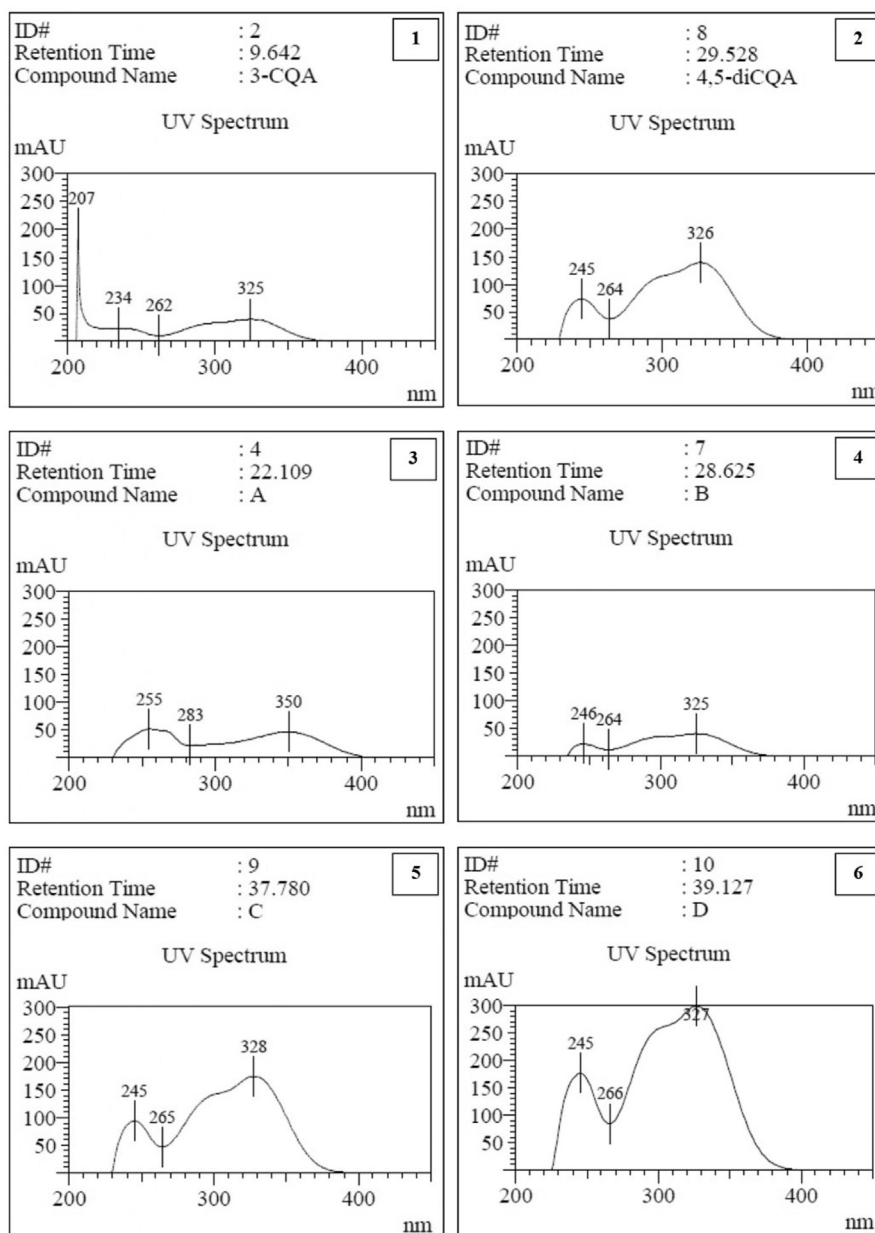


Figure 3. Comparison of UV spectra of some analytes in *P. indica* extract: 3-O-caffeoylquinic acid (1), 4,5-O-dicaffeoylquinic acid (2), unknown analyte A, B, C, and D (3–6), which were analyzed using high-performance liquid chromatography with a photodiode array detector.

Table 6. The effect of gamma irradiation on the antioxidant activity of *P. indica* leaf extract.

Irradiation dose (kGy)	DPPH*	ABTS*	FRAP*
	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)	g Fe ₂ SO ₄ equivalent/100 g extract
0.0	16.83 ± 0.052 ^d	24.77 ± 0.121 ^a	94.56 ± 0.052 ^c
2.5	20.07 ± 0.155 ^b	21.27 ± 0.105 ^c	97.89 ± 0.202 ^d
5.0	18.08 ± 0.116 ^c	19.44 ± 0.129 ^d	107.88 ± 0.189 ^b
7.5	22.29 ± 0.226 ^a	21.70 ± 0.154 ^b	102.18 ± 0.194 ^c
10.0	15.54 ± 0.184 ^e	19.65 ± 0.093 ^d	102.84 ± 0.301 ^c
Ascorbic acid	6.33 ± 0.050 ^f	5.36 ± 0.05 ^e	2798.82 ± 6.417 ^a

Different letters within the same column denote significant differences between groups at $P < 0.05$.

* Data represented as mean ± standard deviation (n = 3).

Meanwhile, the FRAP method evaluates the ability of antioxidants to reduce Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron). The Fe^{3+} -TPTZ complex turns into the intense blue-colored Fe^{2+} -TPTZ, which is quantified by measuring the absorbance at $\lambda 593$ nm (Alam et al., 2013; Dontha, 2016; Opitz et al., 2014; Romulo, 2020; Xiao et al., 2020).

Gamma irradiation significantly affects the antioxidant activity of *P. indica* leaf extract ($P < 0.05$), as shown in Table 6. In the DPPH and ABTS methods, antioxidant capacity is presented as the IC_{50} value, the concentration required to inhibit 50% of radical DPPH \cdot and ABTS \cdot^+ . A sample with a low value of IC_{50} shows a high antioxidant capacity and vice versa. However, in the FRAP assay, antioxidant capacity is evaluated as the amount of Fe^{2+} formed. A higher absorbance shows that a higher amount of Fe^{2+} is present and indicates a stronger reducing ability. In the DPPH method, compared to the control, the antioxidant activity decreased significantly at 2.5, 5, and 7.5 kGy but was significantly increased at 10 kGy. These results differ from the ABTS and FRAP assays, where all irradiation doses (2.5–10 kGy) significantly raised the antioxidant activity. However, the increase in antioxidant activity was not linear with increasing irradiation dose. In the ABTS method, both 5 and 10 kGy showed higher antioxidant activity than other irradiation doses. Meanwhile, in the FRAP method, the 5 kGy dose showed the highest antioxidant capacity.

There was a discrepancy in the antioxidant results between the DPPH assay and the ABTS and FRAP methods, likely because the antioxidant capacity of the compounds was affected not only by their chemical structure but also by other factors, such as the levels of specific compounds, solvent used, pH, and reaction time (Uranga et al., 2016).

Both the irradiated and nonirradiated *P. indica* leaf extracts exhibited IC_{50} values less than 50 $\mu\text{g}/\text{mL}$, indicating that all samples had strong antioxidant capacities, similar to the antioxidant assay performed by Vongsak et al. (2018). It could be that CQAs were the main component responsible for the antioxidant capacity of *P. indica* leaf extract. CQAs are known to have antioxidant activity due to hydroxyl groups on the caffeoyl moiety, which act as free radical scavengers, leading to the breakage of radical chain sequences. This mechanism may act through the hydrogen atom transfer (HAT) and electron transfer (ET) pathways. In HAT, a hydrogen atom from a CQA is abstracted by oxygen-derived radicals, generating relatively more stable CQA-derived radicals, whereas in ET, an electron is initially transferred, followed by a proton (Dontha, 2016; Li et al., 2018; Naveed et al., 2018; Opitz et al., 2014; Uranga et al., 2016). Tošović et al. (2017) suggested that HAT or radical adduct formation (RAF) were possible antioxidative mechanisms of chlorogenic acid (3-CQA) in an acidic or neutral environment. However, in basic media, sequential proton loss ET is likely to occur at a high rate. Besides the HAT and ET pathways, Li et al. (2018) reported that Fe^{2+} chelating was also involved as a diCQA antioxidant mechanism, but RAF was not.

In our study, the antioxidant activity of the irradiated samples was higher than that of the nonirradiated sample. This is because the amount of CQAs, especially diCQAs, increased in the irradiated samples. Xu et al. (2012) reported that diCQAs have a higher antioxidant capacity than

monoCQAs because they have more hydroxyl groups. MonoCQA isomers demonstrate a similar antioxidant capacity, showing no effect from the esterification position on the quinic moiety. Li et al. (2018) reported that the antioxidant abilities of 4,5-diCQA and 3,4-diCQA were higher than that of 3,5-diCQA. Two adjacent caffeoyl moieties make the configuration of 3,4-diCQA and 4,5-diCQA very crowded. Consequently, the molecular energy increases, and the redox potential is raised, thereby elevating its antioxidant capacity. Meanwhile, the caffeoyl moieties in 3,5-diCQA are less crowded, decreasing its molecular energy and redox potential, subsequently lowering the antioxidant capacity.

Our study is in line with other studies that observed that gamma irradiation enhanced the antioxidant activity of *Prunus persica* fruit (Hussain et al., 2010), Malaysian honey (Hussein et al., 2011), Serbian soybeans (Popović et al., 2013), *Ziziphus mauritiana* leaves (Khattak and Rahman, 2016), mulberry leaf extract (Cho et al., 2016), *Arthrospira (Spirulina) platensis* (Shabana et al., 2017), persimmon leaf extract (Cho et al., 2017), black soybean extract (Krishnan et al., 2018), Sakouti and Bondoky dry date fruits (El-Beltagi et al., 2019), *Gnetum gnemon* seeds (Syahdi et al., 2019), and mugwort extract (Hwang et al., 2021). The increase in antioxidant activity is most likely caused by an increase in the content of phenolic compounds or other antioxidative compounds.

3.4. Influence of gamma irradiation on the microbial load

The TAPC and TYM in the irradiated *P. indica* samples were significantly reduced compared to the control ($P < 0.05$) (Table 7). Doses of 2.5–10 kGy effectively reduced the TAPC by approximately one to three logarithmic cycles; the reduction was greater with increasing irradiation doses. Meanwhile, the TYM was drastically decreased to below the detection limit (\log_{10} counts < 1) at 2.5–10 kGy. The TAPC and TYM at 3 months after irradiation showed no difference from immediately after irradiation because the specimens have low water activity and were stored in a glass bottle with low humidity.

Gamma irradiation eradicates microbes through both direct and indirect mechanisms. Photon energy from gamma rays can directly break the DNA of microorganisms, leading to the inhibition of cell division. Photons also cause molecular changes that lead to cell death or the inability of microorganisms to reproduce. Indirectly, gamma irradiation initiates water radiolysis, generating ROS. These free radicals, especially $\cdot\text{OH}$ radicals, cause oxidative damage to the macromolecules of the cell, such as the nucleotide bases of DNA, lipids, and proteins. Approximately 70%–80% of microbial DNA damage by gamma irradiation is caused by ROS formed during water radiolysis. Meanwhile, DNA damage due to the direct absorption of gamma ray energy is only approximately 20%–30%. Therefore, the indirect effect plays a more critical role in microbial cell death than does the direct effect (Aquino, 2012; Gudkov et al., 2019; Munir and Federighi, 2020; Reisz et al., 2014).

In this study, the irradiation dose needed to reduce the TAPC was higher than that needed to reduce the TYM. Similarly, Mun'im et al. (2017) reported that 10 kGy was required to eradicate bacteria in *Peperomia pellucida*, which was higher than the dose needed for the

Table 7. The effect of gamma irradiation on the microbial load of *P. indica* leaf powder.

Irradiation Dose (kGy)	TAPC (\log_{10} CFU/g) [#]		TYM (\log_{10} CFU/g) [#]	
	0 months	3 months	0 months	3 months
0.0	3.83 ± 0.05^a	3.62 ± 0.01^a	3.00 ± 0.07^a	3.01 ± 0.08^a
2.5	2.76 ± 0.04^b	2.62 ± 0.04^b	$<1^b$	$<1^b$
5.0	1.77 ± 0.07^c	1.86 ± 0.09^c	$<1^b$	$<1^b$
7.5	$<1^{*d}$	$<1^d$	$<1^b$	$<1^b$
10.0	$<1^d$	$<1^d$	$<1^b$	$<1^b$

Different letters within the same column represent significant differences between the doses ($P < 0.05$).

[#] Values are presented as the mean \pm SD of triplicate experiments.

^{*} <1 : The estimated aerobic plate count and yeast and mold count were below the detection limit (1 \log_{10} CFU/g).

decontamination of fungi and yeasts (7.5 kGy). Some possible reasons for this are that the initial number of bacteria was higher than the amount of mold and yeast or that radioresistant bacteria were present. *Moraxella sp*, *Deinococcus radiophilus*, and *Deinococcus radiodurans* are examples of radioresistant bacteria that can efficiently repair DNA damage induced by ionizing radiation. Their D10 values (the ionizing radiation dose needed to inactivate 90% of viable colonies or by one logarithmic cycle) are 2–8 kGy, higher than the D10 of commonly vegetative forms of bacteria (0.01–1 kGy). Additionally, *D. radiodurans* has a high Mn/Fe ratio, which results in better adaptability to stress conditions, because manganese ions act as antioxidants, strengthening the enzymatic defense systems against oxidative stress caused by gamma irradiation (Munir and Federighi, 2020).

4. Conclusion

This study revealed that gamma irradiation did not affect either the physical appearance or extract yield of *P. indica* leaf. However, doses of 5–10 kGy significantly increased the caffeoylquinic acid derivatives content. Doses of 2.5–10 kGy caused significant enhancements of antioxidant activity and reduction of microbial load. Moreover, the dose of 10 kGy yielded the best results. Therefore, 10 kGy of gamma irradiation was found to be more effective than other doses in enhancing CQA content and antioxidant activity as well as reducing the microbial load of *P. indica* leaf. Studies should be conducted to further investigate the effect of gamma irradiation on other bioactive compounds and biological activities of *P. indica* leaf during storage time to get more comprehensive results and optimum irradiation dose.

Declarations

Author contribution statement

Ernawati: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abdul Mun'im and Herman Suryadi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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