Effects of irradiation on antioxidant and antimicrobial activities of *Coscinium fenestratum* (Goetgh.) Colebr.

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Abstract. This study aims to investigate the effects of gamma irradiation on antimicrobial activities (against *Staphylococcus aureus* and *Escherichia coli*) and antioxidant activities of *Coscinium fenestratum* (Goetgh.) Colebr., known in Thai as Haem. Gamma irradiation was applied to powder samples of Haem at 5, 10, 15 and 20 kGy. The 60% ethanolic extracts of irradiated and non-irradiated samples were prepared to evaluate total phenolic contents (TPC), antioxidant and antimicrobial activities. Results indicated that TPC of irradiated and non-irradiated samples was not significantly different ($p<0.05$), as the TPC values of all samples ranged from 11.39± 1.17 to 11.99±1.10 mgGAE/g. Ferric reducing antioxidant potential and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity significantly increased ($p<0.05$) after gamma irradiation at 20 kGy and 5 kGy, respectively. The antimicrobial activity was done using agar disc diffusion method. Results showed that ethanolic extracts of various samples exhibited antibacterial activities against *S. aureus* as evidence by the clear zone, 16.3 – 17.5 mm, that formed around the disc samples.

1. Introduction

Plants serve as a valuable source of natural products important to human health. In particular, various studies have focused on the use of plants for natural therapies [1] in the last decade. Many medicinal plants have a large amount of antioxidants such as vitamin C, vitamin E, polyphenols and phytochemical substances. Antimicrobial compounds from plants also represent a potentially novel source of antimicrobial substances. The majority of antimicrobial plant compounds are identified as secondary metabolites, mainly being of terpenoid or phenolic biosynthetic origin. The rest are hydrolytic enzymes (glucanases and chitinases) and proteins acting specifically on membranes of invading microorganisms [2]. As an alternative source to the existing antibiotics, there is a crucial need to discover new antimicrobial compounds from various medicinal plants which can be used to treat many infectious diseases, that are known to be resistant to common antibiotics.

*Coscinium fenestratum* commonly found in Western Ghats of India and Sri Lanka, *C. fenestratum* belonged to the family *Menispermaceae*, is known as Haem in Thailand and has been vastly used as traditional medicine. *C. fenestratum* is considered a critically endangered medicinal plant. It has several medicinal values from root to fruit. The chemical constituents of *C. fenestratum* belongs to different classes of alkaloids, some minor alkaloid as well as ceryl-alcohol, saponin, hentriacontane, sitosterol glucoside, palmitic acid, oleic acid that can be isolated from stem and roots of this plant. Among these,
berberine has been reported to be the major and active constituent having numerous biological activities. In the traditional medicine system, the plant has been used to treat diabetes mellitus and is known have active ingredients with diverse therapeutic purposes. For example, the stems of *C. fenestratum* have antimicrobial, antidiabetic, anti-inflammatory, antioxidants properties that are used to cure various diseases.

Nowadays, the use of herbs and spice as a nutritional resource to improve human health has gained considerable attention. Therefore, it is of important interest to extend the shelf-life of herbs and plants, and also the preservation methods to reduce the microbial contamination. The contamination can occur from the plants themselves, soil, water, air and dust during post-harvest and also during the processing steps. The high microbial loading can cause serious foodborne illness [3]. Therefore, decontamination of plant products is necessary to increase the safety of medicinal plants. The use of ionizing radiation has proven to be an efficient method to diminish microbial load in raw herbs and spice products. However, there remain issues on the influence of irradiation on the active ingredient and phytochemical properties of *C. fenestratum*. Hence in this study, the effect of gamma irradiation on *C. fenestratum* was investigated to establish the optimal irradiation dose that could effectively reduce microbial loading while maintaining antimicrobial and antioxidant activities.

### 2. Materials and Methods

#### 2.1. Source of samples

Dried ground Haem (*C. fenestratum*) was purchased from local distributor in Thailand. Samples of this herb were irradiated at Thailand Institute of Nuclear Technology (Public Organization) using a gamma chamber 5000 (BRIT, India) at the dose rate of 2.96 kGy/h for total doses of 5, 10, 15 and 20 kGy. Non-irradiated samples were also prepared as a reference.

#### 2.2. Antimicrobial activities

##### 2.2.1. Preparation of Plant Extracts

About 6 g of irradiated and non-irradiated ground Haem were soaked in 60 mL of 60% ethanol for 24 h and then filtered through Whatman filter paper No.4 to attain a clear filtrate. The filtrates were evaporated at 55 °C under reduced pressure using rotatory vacuum evaporator and dried by using freeze dryer.

##### 2.2.2. Bacterial strains

The antibacterial potency of irradiated and non-irradiated Haem extracts were evaluated using Gram positive and Gram negative bacterial strains (*S. aureus* and *E. coli*). The bacterial strains were kindly provided by Nopparatratrajathanee Hospital.

##### 2.2.3. Preparation of Inoculums

Each bacterial strain was cultured overnight at 35 °C in Tryptic Soy Broth. The bacterial growth was harvested and diluted to attain viable cell count of 10^7 CFU/mL using spectrophotometer at wavelength of 550 nm.

##### 2.2.4. Antimicrobial activity of plants extract

The agar disc diffusion method is used to evaluate antimicrobial activity of the plants extracts. The plant extract residues (100 mg) were re-dissolved in 1.0 mL of dimethylsulfoxide (DMSO), sterilized through Millipore filter (0.22 µm) and finally a 20 µL drop of the extracts was added on sterilized filter paper discs (6 mm in diameter). Twenty milliliters of Mueller-Hilton agar medium was poured into sterile petri dishes that had been previously inoculated the bacterial suspension. Sterilized filter paper discs inoculated with irradiated and non-irradiated plant extract at concentration of 100 mg/mL were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 20 µL of iodine solution was used as positive control. The plates were incubated at 35 °C for 24 h. The presence of inhibition zones were measured by Vernier Calipers, recorded and considered as indication for antimicrobial activity.
2.3. Total phenolic content (TPC) Analysis
The content of reduced components (expressed in a form of TPC) was estimated using the Folin-Ciocalteu assay according to a method developed by Velioglu et al.[4]. Briefly, a 0.75 mL of 10-fold diluted Folin-Ciocalteu reagent and 100 µL of 60% ethanolic extract were mixed in a test tube. The mixture was left at room temperature for 5 min. Then, 0.75 mL of 6% (w/v) sodium carbonate solution was added to the thoroughly mixed solution. Then the mixture was homogenized and left at room temperature for 90 min. TPC was determined using a spectrophotometer at absorbance wavelength of 725 nm. The standard calibration curve was plotted using gallic acid at the concentration of 0.02-0.1 mg/mL. The TPC was expressed in terms of gallic acid equivalent (GAE) mg/g.

2.4. Determination of free radical scavenging activity (DPPH)
The antioxidant activity was investigated using the method previously described by Khattak et al. [5] with a slight modification. It was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The 100 µL of 60% ethanolic extract was added to 900 µL of DPPH in methanol solution (150 µM) in a test tube and shaken vigorously. After incubation at room temperature for 15 min in the dark, the absorbance of each solution was determined at 517 nm. The free radical-scavenging activity was expressed in the form of half maximal inhibitory concentration (IC50, mg/mL).

2.5. Determination of ferric reducing antioxidant potential (FRAP)
FRAP assay was performed according to the method of Benzie and Strain [6]. The FRAP reagent was prepared by mixing 16.7 mM FeCl3•6H2O and 8.3 mM 2,4,6-tripyridyl-s-triazine (TPTZ) with 250 mM acetate buffer, pH 3.6. A solution of 75 µL of 60% ethanolic extract and 225 µL of distilled water were added to 2.25 mL of freshly prepared FARP reagent in a test tube. The mixture was incubated at room temperature throughout the reaction. The absorbance was read at initial and after 30 min at 596 nm using UV-vis spectrophotometer during the monitoring period. The antioxidant potential of the samples were determined based on a calibration curve plotted using FeSO4•7H2O at concentration ranging between 400 and 2000 µM.

2.6. Statistical analysis
All measurements were obtained in triplicates measurements and results were expressed as mean ± standard deviation. The Statistical Package for Social Sciences (SPSS) for Windows version (21) was used to analyse the data. Statistical significance was declared at p< 0.05.

3. Results and discussion
Dried ground Haem herb samples were exposed to gamma irradiation at the doses of 5, 10, 15 and 20 kGy. The effect of irradiation on antimicrobial and antioxidant activities were evaluated for all the irradiated and non-irradiated samples.

3.1. Antimicrobial activities
The antimicrobial activities of irradiated and non-irradiated Haem extracts were assayed in vitro by agar disc diffusion method against S. aureus and E. coli (Table 1). The results indicated that both irradiated and non-irradiated Haem extracts exhibited similar antimicrobial activity against S. aureus alone, but not E. coli. After irradiated up to 20 kGy, the diameter of inhibition zone was not significantly different between irradiated and non-irradiated samples. The highest antimicrobial activity against S. aureus was obtained in samples that had been irradiated at 20 kGy as evidence by formation of clear zone with diameter 17.55±0.69 mm compared to the positive control (Betadine, 10.66±0.33 mm) as shown in Figure 1. Irradiated samples showed the tendency of increased antimicrobial activities. The results indicated that Haem can be used for developing antimicrobials against S. aureus. This results are in agreement with previous research of Khattak et al. [7], which indicated that gamma irradiation up to 10 kGy did not effected on the antimicrobial activities of the Nigella sativa seed. In addition, another
study conducted by Khattak and Simpson [8] indicated that the radiation treatment with the dose of 25 kGy improve the antibacterial activity of *Glycyrrhiza glabra* extract against *Micrococcus luteus*.

### Table 1. Agar disc diffusion assay of Haem extracts against *S. aureus* and *E. coli*

| Dose (kGy) | Zone of inhibition (mm) of extracts against *S. aureus* | Zone of inhibition (mm) of extracts against *E. coli* |
|------------|--------------------------------------------------------|-----------------------------------------------------|
| 0          | 16.88±0.69 a                                          | 0                                                   |
| 5          | 17.00±0.66 a                                          | 0                                                   |
| 10         | 17.11±0.69 a                                          | 0                                                   |
| 15         | 16.44±0.69 a                                          | 0                                                   |
| 20         | 17.55±0.69 a                                          | 0                                                   |

*Means in the same column with different alphabetical letters are significantly different (p<0.05)*

![Figure 1](image)

**Figure 1.** Inhibition zone of Haem extracts of (a) non-irradiated (b) 5 kGy (c) 10 kGy (d) 15 kGy (e) 20 kGy (f) Betadine (positive control) against *S. aureus*.

### 3.2. Total phenolic contents (TPC), free radical scavenging activity (DPPH) and ferric reducing antioxidant potential (FRAP)

The effects of gamma irradiation in terms of TPC, FRAP and DPPH of ground Haem extracts were summarized in Table 2. In this present study there was a no significant increase in TPC of all irradiated samples when compared to non-irradiated sample, the value varied between 11.39-11.99 mg GAE/g. These results were correlated with previous studies in which the gamma irradiation could increase the phenolic content in soybeans [9]. The increasing of TPC could be attributed to the release of phenolic compound from glycosidic components and the degradation of the larger phenolic compounds into smaller ones by gamma irradiation as suggested by Harrison and Were [10] which was consistency with this study. In addition, the work by Adamo et al. [11] suggested that the destructive process of oxidation and gamma irradiation are capable of breaking the chemical bonds of polyphenols and therefore allowing the release of soluble phenols that are presented in the form of low molecular weights.

The free radical scavenging activity of *C. fenestratum* was evaluated by its ability to reduce DPPH, a stable free radical. DPPH was reported by its half maximal inhibitory (IC$_{50}$, mg/mL). The ethanolic extracts of irradiated samples showed more antioxidant activities than non-irradiated ones. Higher dose of irradiation resulted in a significantly increase in DPPH value, which was ranged from 3.97±0.40 to 4.61±0.13 mgAAE/g. Our present study was also well in accordance with the previous study by Jo et al. [12] that reported the doses between 10 and 20 kGy applied to ethanol extracts of green tea leaves could raise DPPH radical-scavenging ability immediately after treatment. Irradiation at 20 kGy was found to increase the radical-scavenging ability of phytic acid to a maximum [13]. On the other hand, Lampart-Szczapa et al. [14] reported that the antioxidant decreased after increasing the doses in lupin seed extracts. Harmonized with Suhaj et al. [15] reported that the DPPH scavenging activities of black pepper significantly decreased after irradiation at 5, 7.5, 10, 20 and 30 kGy.

The antioxidant potential of the control and irradiated samples were estimated from their ability to reduce the TPTZ-Fe(III) complex to the TPTZ-Fe(II) complex. The values of FRAP at various doses fluctuated between 53.56 and 56.56 µmol FeSO$_4$/g. The results also revealed that antioxidant activity in term of FRAP was not significantly increased in irradiated samples (5-15 kGy). According to previous
study of Korean red ginseng powder by Byun et al. [16], it was found that hydrogen donating activity was not significantly changed after irradiated up to 10 kGy. Kitazura et al. [17] reported that the 5-25 kGy of gamma irradiation did not show any effect on the antioxidant potential of cinnamon compounds. On the other hand, Khadijeh et al. [18] reported that irradiation at the doses of 2 and 6 kGy caused a decrease (p<0.05) in FRAP values of H2 extracts compared to the control. However, there was no difference in FRAP value of irradiated H2 at 10 kGy compared to the control. This data showed that higher irradiation maintained the antioxidant activity of almond hull. Evidently, gamma irradiation would enhance the antioxidant activity in some foods, while in others the antioxidant activity was reduced. However, no general pattern of gamma irradiation on antioxidant activity was found.

### Table 2. Total phenolic content, DPPH and FRAP value of irradiated and non-irradiated Haem.

| Dose(kGy) | Total phenolic content* (mgGAE/g) | DPPH* (mgAAE/g) | FRAP* (µmol FeSO₄/g) |
|-----------|----------------------------------|-----------------|----------------------|
| 0         | 11.39 ± 1.17 a                    | 3.97 ± 0.40 c   | 53.56 ± 1.34 b       |
| 5         | 11.58 ± 0.95 a                    | 4.61 ± 0.13 a   | 54.25 ± 1.84 b       |
| 10        | 11.63 ± 0.93 a                    | 4.48 ± 0.22 ab  | 54.29 ± 1.26 b       |
| 15        | 11.64 ± 0.86 a                    | 4.22 ± 0.25 bc  | 54.66 ± 1.56 b       |
| 20        | 11.99 ± 1.10 a                    | 4.35 ± 0.34 ab  | 56.56 ± 1.49 a       |

*Means in the same column with different alphabetical letters are significantly different (p<0.05)

### 4. Conclusion

Gamma irradiation has been well established as a method to diminish microbial load in raw materials. In this study, the results indicated that irradiation up to 20 kGy neither significantly change the antimicrobial activity nor the total phenolic contents of *C. fenestratum*. The data showed significant increase in antioxidant activities in term of DPPH value at higher doses, which may be beneficial for plant antioxidant activities. Meanwhile, FRAP value was significantly increased after irradiated at 20 kGy. This investigation suggests that radiation treatment up to 20 kGy is safe and beneficial for *C. fenestratum*.

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