Effects of gamma ray and electron beam irradiation on reduction of microbial load and antioxidant properties of Chum-Hed-Thet (Cassia alata (L.) Roxb.)

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Abstract. Considering the growing demands of herbal medicines, Cassia alata (L.) Roxb. has been reported to have various phytochemical activities. It has also been called in Thai as Chum-Hed-Thet. In this study, C. alata (L.) Roxb. powder were exposed to gamma and electron beam irradiation at doses of 0, 5, 10, 15 and 20 kGy. At the dose of 10 kGy, both of gamma and electron beam irradiation were sufficient in reducing microbial load of irradiated samples as specified in Thai pharmacopoeia (2005). These include the total aerobic microbial count of $< 5.0 \times 10^3$ CFU/g, total fungi count of $< 5.0 \times 10^3$ CFU/g, bile tolerant gram negative bacteria of $< 10^3$ (per g). In addition, pathogenic Clostridium spp. (per 10 g), Salmonella spp. (per 10 g), S. aureus (per 1 g) and E.coli (per 1 g) were absence. In terms of the bioactive molecules, the total phenolic content, DPPH free radical scavenging activity and ferric reducing antioxidant potential of unirradiated and irradiated samples were 19.32-22.44 mg gallic acid equivalent/g, 5.20-7.82 mg ascorbic acid equivalent/g and 69.46-82.06 µmol FeSO₄/g, respectively. However, there were no significant differences between unirradiated and irradiated samples ($p > 0.05$). Therefore, both of radiation by gamma ray or electron beam at 10 kGy was sufficient in elimination of microbial flora and did not significantly affected the total phenolic content and antioxidant activities of C. alata (L.) Roxb.

1. Introduction

Cassia alata (L.) Roxb. is commonly known in Thai as Chum-Hed-Thet. The common names are candle bush, ringworm bush and Christmas candle. This plant is widely found in tropical regions, sometimes cultivated for medicinal purposes. The main parts used as herbal medicine are the leaves, the barks and the roots. They are very high medicinal values like antimicrobial property and antioxidant activity and traditionally being used in the treatment of skin infections in man. Leaf extract is a good antioxidant and the compound obtained from has been identified as a flavonol compound and named as Kaempferol. Due to its origin it is generally highly contaminated by microorganisms. While the decontamination technologies for herbs and spices, like ethylene oxide, thermal inactivation or the use of steam have disadvantages. The fumigation by ethylene dioxide is prohibited in Europe due to carcinogenic potential in human. However, herbal substances are heat sensitive products, thus the application of steam can cause alteration of aroma and odor, as well as the reduction of volatile compounds. The application of gamma irradiation for the decontamination is a quite efficient process. Recently, electron beam irradiation has been introduced as an alternative method, as though that it being environmental friendly and rapid method for controlling spoilage of foods and improve the hygienic qualities of pharmaceutical and agricultural products.
The aim of this work was to study the potential application of gamma ray and electron beam irradiation in order to investigate the effects on the microbial contamination, the total phenolic content and antioxidant activity of C. alata (L.) Roxb. powder.

2. Materials and methods

2.1. Materials
Powder of C. alata (L.) Roxb. was purchased from retailer for this present study, which was procured from Sub-Central Thailand region, in March to April, 2014. One hundred grams of dry samples were packed and sealed in aluminium foil packets. Ten packets of dry samples was placed in polyethylene bags and stored at ambient temperature.

Figure 1. Flowers and leaves of Chum-Hed-Thet (C. alata (L.) Roxb.).

2.2. Gamma ray and electron beam irradiation
Gamma ray and electron beam irradiation were performed at Gems Irradiation Centre, Thailand Institute of Nuclear Technology (Public Organization), Ongkharak district, Nakhon nayok province, Thailand. Samples (1,000 g) were exposed to radiation at various doses of 5, 10, 15 and 20 kGy. Gamma irradiation were carried out by gamma irradiator from source of Co-60 model Type II (ANS N43.10.), Power Plus System Ltd., UK. Electron beam irradiation were carried out by electron beam accelerator at energy of 5.3 MeV, model 20/16, Mevex, Canada. The unirradiated samples served as controls.

2.3. Microbial analysis
The microbial study was done to enumerate the total aerobic microbial count (CFU/g), total fungi count (CFU/g). Specific microorganisms such as bile tolerant gram negative bacteria (per 1 g), pathogenic Clostridium spp.(per 10 g), Salmonella spp. (per 10 g), Staphylococcus aureus (per 1 g) and Escherichia coli (per 1 g) were determined. Control and the irradiated samples were kept at ambient temperature until the analysis were carried out. The samples were taken after 5 days of irradiation. All test were run in triplicate samples and mean values were used. The level of microbial contaminants of unirradiated and irradiated samples were analyzed by method of microbial limit test indicated in Thai pharmacopoeia (2005)[6].

2.4. Total phenolic content and antioxidant analysis

2.4.1. Sample preparation. The samples were taken after 7 days of irradiation. 0.1 g of samples were extracted for 1 h at room temperature in ultrasonic bath with 10 ml of methanol. The sample suspensions were centrifuged, and the supernatant was filtered and the filtrate stores at -20 °C until analysis.
2.4.2. **Total phenolic content.** The content of reducing components was estimated using the Folin-Ciocalteau assay according to the method developed by Velioglu et al., 1998[7]. The total phenolic content was expressed as gallic acid equivalent (GAE) mg/g of dry sample. The estimation of the phenolic compounds was carried out in triplicate.

2.4.3. **Free radical scavenging assay with DPPH.** This assay was modified from that described in a previous report by Khattak et al., 2008[8] with slight modifications. All test were run in triplicate. The absorbance of each solution was determined by spectrophotometer at 517 nm. The free radical scavenging activity was expressed as ascorbic acid equivalent.

2.4.4. **Ferric reducing potential (FRAP).** Ferric reducing potential (FRAP) assay was performed according to the method described by Benzei and Strain, 1996[9]. The absorbance was read at 596 nm. using a spectrophotometer. The antioxidant potential of the samples was analyzed based on a calibration curve plotted using FeSO\(_4\)·7H\(_2\)O at concentrations ranging from 400 to 2,000 μM. All test were run in triplicate and mean values were used to calculate concentration of an antioxidant. A ferric reducing ability was expressed as μmol FeSO\(_4\)/g.

2.4.5. **Statistical analysis.** Data (2.4.2, 2.4.3 and 2.4.4) were analyzed by one-way ANOVA and differences among treatment means were determined by Duncan’s new multiple-range test.

3. **Results and Discussion**

3.1. **Microbial analysis**

As show in table 1, the initial levels of total aerobic microbial count (TAMC) and the total fungi count of *C. alata* (L.) Roxb. in this present study were quite high at the levels of 7.60 x 10\(^6\) and 1.10 x 10\(^7\) CFU/g, respectively. The results showed that the initial microorganisms levels were exceed the acceptable limits for microbial contamination as indicated in Thai pharmacopoeia (2005) (TAMC < 5.0x10\(^5\) CFU/g, total fungi count < 5.0x10\(^4\) CFU/g, not more than 10\(^5\) bile tolerant gram negative bacteria (per 1 g) and specific microorganisms were absence). Gamma ray and electron beam irradiation at dose of 10, 15 and 20 kGy was effective in reduction of the initial of total aerobic microbial count by decreased 2-5 log cycle and eliminated total fungi count to less than 10 CFU/g in irradiated samples. The results indicated that the gamma ray and electron beam irradiation significantly reduced microbial load in samples in dose dependent manner. For specific microorganisms, gamma ray and electron beam irradiation at dose of 5 kGy completely eliminated bile tolerant gram negative bacteria to less than 10 (per 1g), whereas *Salmonella* spp. (per 10g), pathogenic *Clostridium* spp. (per 10g), *S. aureus* (per 1 g) and *E. coli* (per 1 g) were not found in unirradiated and irradiated samples.

These results indicated that gamma ray and electron beam irradiation at dose of 10 kGy was sufficient in reduction of microbial contamination of *C. alata* (L.) Roxb. as indicated in Thai pharmacopoeia(2005). However, in this present study, dosage of greater than 20 kGy was needed to completely decontaminated of *C. alata* (L.) Roxb.

The effects of gamma ray and electron beam irradiation on the microbial contamination of herbs are dependent on dose and the type of microorganism present. The effects on bacterial reduction are relatively dose dependent manner, so the end result depends on the initial contamination level[10][11].

Phianphak et al. 2007 reported seventeen species of herbs established in Thai traditional remedies were microbially decontaminated by gamma radiation doses of 7.7 and 8.8 kGy. All year round post-harvested and powdered herbs in the study were contaminated with microorganisms, as shown by total bacteria count in the range of 10\(^2\)-10\(^5\) CFU/g and mold counts in the range of 10\(^2\)-10\(^5\) CFU/g. *Staphylococcus* spp.10\(^2\)-10\(^5\) CFU/g, *Salmonella* spp.10\(^2\)-10\(^5\) CFU/g and coliform bacteria 10\(^3\)-10\(^7\) CFU/g. No microorganisms were found after gamma treatment[12]. Eamsiri et al. 2016 reported the initial microorganisms of unirradiated sample of *Butea Superba*. Roxb. (Kwao Khrua Dang) root extract were exceed the permissible limits of world health organization (WHO). The results of total
viable bacterial counts indicated that the non-irradiated samples of Kwao Khrua Dang powder were highly contaminated with bacteria at the level of 3.9x10^6 CFU/g. Electron beam irradiation at doses of

Table 1. Microbial analysis of C. alata (L.) Roxb. powder irradiated at varied doses of gamma ray and electron beam irradiation.

| Microbial Flora                        | Type      | Dose (kGy) |
|---------------------------------------|-----------|------------|
|                                       |           | 0          | 5          | 10         | 15         | 20         |
| TAMC (CFU/g)                          | Gamma     | 7.60 x 10^6 | 5.30 x 10^7 | 4.20 x 10^7 | 1.0 x 10^7 | 10         |
|                                       | E-beam    | 5.60 x 10^7 | 2.40 x 10^7 | 2.0 x 10^7  | 10         |
| Total fungi count (CFU/g)             | Gamma     | 1.10 x 10^7 | < 10        | < 10        | < 10        | < 10        |
|                                       | E-beam    | 1.4 x 10^7  | 10          | < 10        | < 10        | < 10        |
| Bile tolerant gram negative bacteria  | Gamma     | Less than 10^3 | < 10       | < 10        | < 10        | < 10        |
|                                       | E-beam    | more than 10^3 | < 10      | < 10        | < 10        | < 10        |
| Pathogenic *Clostridium* spp. (per 10 g) | Gamma   | NF         | NF         | NF         | NF         | NF         |
|                                       | E-beam    | NF         | NF         | NF         | NF         | NF         |
| *Salmonella* spp. (per 10 g)          | Gamma     | NF         | NF         | NF         | NF         | NF         |
|                                       | E-beam    | NF         | NF         | NF         | NF         | NF         |
| *S. aureus* (per 1 g)                 | Gamma     | NF         | NF         | NF         | NF         | NF         |
|                                       | E-beam    | NF         | NF         | NF         | NF         | NF         |
| *E. coli* (per 1 g)                   | Gamma     | NF         | NF         | NF         | NF         | NF         |
|                                       | E-beam    | NF         | NF         | NF         | NF         | NF         |

All test were run in triplicate samples and mean values were used. NF: Not Found.

5 kGy reduced the microbial contamination to the standard level, complete microbial decontaminations were obtained at 10 kGy irradiation. Irradiated doses of 5, 10, 15 and 20 kGy did not affect total phenolic content and antioxidant activity [13]. Prajna et al, 2012 reported electron beam irradiation at dosage of 8 kGy was sufficient to destroy all fungal contaminants in six different herbal raw materials whereas dosage of 12 kGy was needed to completely hygienise. Doses selected in the study were not affected the nutritional quality like carbohydrates, proteins, phenolics and tannins of herbs to a larger extent [10].

3.2. Total phenolic content and antioxidant activity

As show in table 2, the total phenolic content, the DPPH free radical scavenging activity and the ferric reducing antioxidant potential (FRAP) of unirradiated and irradiated samples exhibited ranges from 19.32-22.44 mg gallic acid equivalent/g, 5.20-7.82 mg ascorbic acid equivalent/g and 69.46 to 82.06 μmol FeSO₄/g, respectively. The results indicated that no significant differences in the phenolic content, antioxidant activities by DPPH and the ferric reducing antioxidant potential (FRAP) between the un irradiated and irradiated samples of gamma ray and electron beam irradiation at doses of 5, 10, 15 and 20 kGy (p > 0.05).

This results according to the other studies, gamma irradiated at 1, 3, 5 and 10 kGy did not show significant differences in antioxidant activity compared with the unirradiated of seven dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg and vanilla) [14] and tea (Camellia sinensis) [15]. Gamma irradiation of sage and oregano at 30 kGy did not have any significant effect on DPPH radical scavenging activity, reducing power and total phenolic content (p>0.05) [16]. Gamma radiation processing at doses of 5 kGy complete decontamination of microorganisms in Triplala (a mixture of Emblica officinalis, Terminalia chebula and Terminalia bellirica) and irradiated samples showed linearly increasing concentration of gallic acid (3.3 to 4.5 times), total phenolic contents (2.16 to 2.87 times) and antioxidant properties with increasing radiation dose up to 25 kGy [17]. Radiation treatment with 10 kGy and 20 kGy of gamma radiation and electron beam did not affect significantly the yield of extraction of chamomile (Matricaria chamomilla L.) dried powder and sensory qualities of all extracts were not significantly different depending on irradiation types and doses [18].
Table 2. Analysis of total phenolic content, DPPH and FRAP of *C. alata* (L.) Roxb. irradiated at varied doses of gamma ray and electron beam irradiation.

| Ionizing radiation | Dose (kGy) | Total phenolic content (mg gallic acid equivalent/g) | DPPH (mg ascorbic acid equivalent/g) | FRAP (μmol FeSO₄/g) |
|--------------------|------------|-----------------------------------------------------|-------------------------------------|---------------------|
| Gamma              | 0          | 22.08 ± 0.98 ab                                      | 7.21 ± 0.67 ab                      | 82.06 ± 13.30 a     |
|                    | 5          | 21.14 ± 0.84 ab                                      | 6.69 ± 1.37 ab                      | 75.83 ± 1.74 a      |
|                    | 10         | 22.13 ± 1.02 ab                                      | 7.82 ± 0.54 ab                      | 76.21 ± 1.31 a      |
|                    | 15         | 19.32 ± 0.44 a                                      | 6.11 ± 0.73 ab                      | 69.46 ± 0.85 a      |
|                    | 20         | 20.37 ± 1.05 ab                                      | 8.61 ± 0.45 ab                      | 70.74 ± 1.86 a      |
| Electron           | 5          | 22.44 ± 2.42 b                                      | 6.81 ± 1.28 ab                      | 81.53 ± 14.27 a     |
|                    | 10         | 21.22 ± 2.91 ab                                      | 5.64 ± 1.45 a                       | 79.22 ± 13.39 a     |
|                    | 15         | 21.14 ± 1.35 ab                                      | 5.20 ± 1.22 a                       | 79.99 ± 12.14 a     |
|                    | 20         | 21.33 ± 1.47 ab                                      | 5.62 ± 1.82 a                       | 78.31 ± 10.35 a     |

Each value is expressed as mean ± standard deviation (n=3). 
Mean with difference small letters within column attribute are significantly different (*p* < 0.05)

4. Conclusions
The results of this work showed that gamma ray and electron beam irradiation could be considered as effective method to improve the microbial qualities of *C. alata* (L.) Roxb. powder. Both of gamma ray and electron beam irradiation with doses of 10 kGy led to desired microbial reduction with acceptable variation of antioxidant activity, whereas dosage of greater than 20 kGy was needed to completely hygienise. Dose selected in this study at 5, 10, 15 and 20 kGy were no significant differences on total phenolic content and antioxidant activity of *C. alata* (L.) Roxb.

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