

## **Gamma irradiation versus microbial contamination of Thai medicinal herbs**

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### **Abstract**

Phianphak, W., Rengpipat, S. and Cherdshewasart, W.

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Songklanakarin J. Sci. Technol., March 2007, 29(Suppl. 1) : 157-166

Seventeen species of herbs established in Thai traditional remedies were microbially decontaminated by gamma-irradiation doses of 7.7 and 8.8 kGy. The herb samples were randomly collected four times from producers in Chiangmai during a 1-year period. These were tested, qualitatively and quantitatively, for total aerobic bacteria, *Staphylococcus* spp., *Salmonella* spp., coliform bacteria, and fungi before and after gamma treatment. No microorganisms were found after gamma treatment; and the color, aroma, and texture of the herbs remained normal. The applied dose of gamma irradiation was within the regulatory limits in Thailand (<10 kGy) and the main export country (USA < 30 kGy). Gamma irradiation is an effective treatment for microbial decontamination of Thai export herbs.

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**Key words :** Thai herbs, gamma irradiation, microorganism

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## บทคัดย่อ

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การใช้รังสีแกมมากำจัดการปนเปื้อนของจุลินทรีย์ในสมุนไพรไทย  
ว. สงขลานครินทร์ วทท. มีนาคม 2550 29(ฉบับพิเศษ 1) : 157-166

การใช้รังสีแกมมากำจัดการปนเปื้อนของจุลินทรีย์ในสมุนไพรไทยที่นิยมนำมาใช้เป็นส่วนผสมในการผลิตยาแผนโบราณ 17 ชนิด จากตัวอย่างที่สุ่มเก็บจากผู้ผลิตในจังหวัดเชียงใหม่จำนวน 4 ครั้ง ครอบคลุมทุกฤดูตลอดปี เมื่อนำสมุนไพรก่อนและหลังการฉายรังสีขนาด 7.7 และ 8.8 kGy มาตรวจหาชนิดของจุลินทรีย์ได้แก่ *Staphylococcus* spp., *Salmonella* spp., โคลิฟอร์มแบคทีเรีย และรา รวมทั้งปริมาณของจุลินทรีย์ทั้งหมด พบว่าภายหลังการฉายรังสีแกมมา ตรวจไม่พบจุลินทรีย์ใดๆ ในสมุนไพรหลังการฉายรังสี และสมุนไพรที่นำมาตรวจสอบทั้งหมดมีสี กลิ่นและเนื้อสัมผัสคงเดิม ปริมาณรังสีที่ใช้ต่ำกว่าข้อกำหนดในประเทศไทย (<10 kGy) และประเทศคู่ค้าที่สำคัญ(สหรัฐอเมริกา < 30 kGy) การฉายรังสีแกมมาจึงเป็นวิธีการที่ใช้กำจัดการปนเปื้อนของจุลินทรีย์ในสมุนไพรไทยเพื่อการส่งออกได้อย่างมีประสิทธิภาพ

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In a world increasingly concerned with health and longevity, many people, including Thais, show enhanced interest in medicinal plants -- for example herbs, natural plant extracts or preparations -- that have long been used in traditional medicine in Thailand (Farnsworth and Bunyapraphatsara, 1992; Bunyapraphatsara and Chokchaichareonporn, 1996; Saisang, 2005). Some Thai herbs are major agricultural products used traditionally for medicinal treatment and as essential ingredients in several Thai dishes, cosmetics, and animal feed (Saritpan, 1978; Suksrignarm, 1980; Chulalongkorn University, Herbal Medicine Research Study Project, 1980; Chulalongkorn University, Herbal Medicine Research Study Project, 1981; Bunyapraphatsara and Chokchaichareonporn, 1996; Tiengthavaj *et al.*, 1998; Saisang, 2005). Herb products have recently become one of the most popular supplementary foods, with an expansion rate of ~28% in 2004 (Department of Export Promotion, Ministry of Commerce, Thailand, 2005; <http://www.moc.go.th>). It was reported that the 2004 market value of herb and medicinal plants as food supplements and cosmetics in the local market alone reached ~47 million USD (Department of Export Promotion,

Ministry of Commerce, Thailand, 2005; <http://www.moc.go.th>).

Recently, preparation of herbs in dried-powder form has been shown to maintain the plants' properties of aroma, color, and flavor. Reduction of weight in dried-powder herbs also reduces transportation costs. Generally, herbs are valued for their distinctive aroma, color and flavor. Unfortunately, they are often contaminated with high levels of bacteria, molds, and yeasts; if untreated, the herbs will result in rapid spoilage of the foods they are supposed to enhance. Since herbs are often contaminated with pathogenic bacteria, they can also result in serious foodborne illness. Post-harvest and post-processing, herbs are always contaminated with microorganisms from the plants themselves, soil, water, air, and dust. Wide spectrum of microorganisms and microbial loads have been reported previously in medicinal plants (Frazier and Werthoff, 1978; De Boer *et al.*, 1985; McKee, 1995; Kneifel *et al.*, 2002); these microorganisms can later contaminate end products (Czech *et al.*, 2001). Moreover, medicinal plants have to be considered as dietary ingredients, which should be well-prepared under Good Manufacturing Practices (GMPs); this invokes the principle

that the best quality of raw material will yield the better product.

Irradiation results in a much lower level of microbial contamination and is often the only treatment effective enough to meet standards set by processors operating under Hazard Analysis Critical Control Points (HACCP) or International Standards Organization (ISO) standards (Mossel, 1989; Radomyski *et al.*, 1994; Harding and Pinteric, 1995; Farrar IV, 2000; Panisello *et al.*, 2000; Molins *et al.*, 2001). A dose of 5-10 kGy results in an immediate minimum 6-7 log-cycle

reduction of bacterial vegetative cell and 2-3 log-cycle reduction of bacterial spores. Most countries set maximum dose regulatory limits, and these higher limits allow for higher levels of microbial control. Diehl (1995) suggested the use of irradiation as a standard technique in maintaining good quality of herbs. Gamma irradiation in the range of 3-10 kGy reduces the total aerobic viable cell counts in highly contaminated spices and dry herbs to below  $10^3$ - $10^4$  CFU/g (Farkas, 1988). The maximum dose allowance for herb treatment in USA is 30 kGy (FDA, 1986), whereas, no more than 10

**Table 1. Thai medicinal herbs and some effects on human health.**

Scientific name (Thai common name)	Part of plant	Some effects on human health	References
1. <i>Aegle marmelos</i> (Matume)	Fruit	Antidizziness, antihelminthic activity, antidiarrhoea, and thirsty remedy	Lamba and Bhargava, 1969; Nadkarni, 1954;
2. <i>Betula alnoides</i> (Phaya Sua Krong)	Stem	Strength nourishing, treatment fatigue, antimalaise	<a href="http://www.medplant.mahidol.ac.th">http://www.medplant.mahidol.ac.th</a>
3. <i>Bixa orellana</i> (Kum Saad)	Leaves and seeds	Antimicrobial activity, antiemetic, and anti-pruritic	Fleischer <i>et al.</i> , 2003
4. <i>Boesenbergia rotunda</i> (Krachaikwao)	Rhizomes	Anti-inflammation	Tuchinda <i>et al.</i> , 2002
5. <i>Butea superba</i> (Red Kwaokrua)	Tuberous root	Treatment for erectile dysfunction	Cherdshewasart and Nimsakul, 2003
6. <i>Caesalpinia sappan</i> (Phang)	Stem	Analgesic and anti-inflammatory agent; Antihypercholesteremia	Hu <i>et al.</i> , 2003
7. <i>Cyperus rotundus</i> (Huahaowmu)	Leaves and/or roots	Anti- <i>Candida</i> activity, strength nourishing, enhance food digestibility, treatment of beri beri, gastric ulcer, fatigue,	Teixeira Duarte <i>et al.</i> , 2005; <a href="http://www.tropilab.com">http://www.tropilab.com</a> , <a href="http://www.holistic-online">http://www.holistic-online</a>
8. <i>Ficus foveolata</i> (Mah)	Vine	Blood nourishing, anti-toothache, antimalaise	<a href="http://www.medplant.mahidol.ac.th">http://www.medplant.mahidol.ac.th</a>
9. <i>Mesua ferrea</i> (Bunnag)	Flower	Antiprotozoa agents, antibacterial activity, blood nourishing, antimalaise	Verotta <i>et al.</i> , 2004
10. <i>Mimusops elengi</i> (Pikul)	Bark, flower	Anti-ulcer activity, fever rectification, antiheadache, antimalaise	Shah <i>et al.</i> , 2003; <a href="http://www.gpo.or.th/herbal">http://www.gpo.or.th/herbal</a>
11. <i>Mucuna collettii</i> (Black Kwaokrua)	Tuberous root	Strengthen sexual potency	<a href="http://www.dreddyclinic.com">http://www.dreddyclinic.com</a>
12. <i>Phyllanthus emblica</i> (Makampom)	Fruit	Antioxidant activity, voice nourishing, thirsty remedy	Bandyopadhyay <i>et al.</i> , 2000
13. <i>Piper retrofractum</i> (Deeplee)	Fruit	Treatment of bronchial asthma, bronchitis, muscle pains and incurable diseases	<a href="http://www.gpo.or.th/herbal">http://www.gpo.or.th/herbal</a> <a href="http://www.medplant.mahidol.ac.th">http://www.medplant.mahidol.ac.th</a>
14. <i>Pueraria mirifica</i> (White Kwaokrua)	Tuberous root	Treatment for menopausal symptoms	Muangman and Cherdshewasart, 2001
15. <i>Terminalia bellerica</i> (Samor-Pipaek)	Fruit	Anti-diabetic activity	Sabu and Kuttan, 2002
16. <i>Terminalia chebula</i> (Samor-Thai)	Fruit	Anti-diabetic activity; inhibition of cancer cell growth	Sabu and Kuttan, 2002; Saleem <i>et al.</i> , 2002
17. <i>Tinospora crispa</i> (Bohraphet)	Stem	Antihyperglycemia	Noor and Ashcroft, 1998

kGy is allowed for use in Thailand (Regulation and Control in Irradiated Food, Ministry of Thai Public Health, 1986). Cleanliness and aroma properties are considered the most important factors when evaluating the quality of herbs. Thus, this study will aim to apply gamma irradiation to reduce microorganism of Thai medicinal powdered herbs.

### Materials and methods

#### Source of herbs

Seventeen Thai herbs with previously reported beneficial effects on human health (Table 1) were obtained in powdered form from producers in Chiang Mai, Thailand. Herb samples (500 g), chopped and ground into powder, were kept in sterile polyethylene bags at ambient temperature before test. Random samplings were performed four times: in August, 2003 to represent the rainy season; in November, 2003 and January, 2004, to represent the winter season; and in June, 2004 to represent the summer season. A portion of each herb sample was packed in a sterile polyethylene bag at ambient temperature before irradiation.

#### Media for cultivation

Total aerobic bacteria were enumerated on plate count agar (Difco, USA). Mannitol salt agar (Oxoid, USA) was the selective medium for *Staphylococcus* spp.; whereas, *Salmonella* spp. were determined on both Salmonella-Shigella agar (Oxoid, England) and MacConkey agar (Difco, USA). Chromocult coliform agar (Merck, Germany) was used as the selective medium for coliform bacteria. Fungi (mold and yeast) counts were done on potato dextrose agar (Difco, USA) acidified to pH 3.5 with (10%) tartaric acid in order to inhibit bacterial growth.

#### Sample irradiation

Herbs packed in polyethylene bags were irradiated using a cobalt-60 irradiator (JS-8900, Canada) with the source strength of ~150,000 Ci. The doses, measured by nylon thin film FWT-60-00 dosimetry (USA), were 7.70 kGy in the first trial

and 8.80 kGy in the remaining three experiments. The initial minimum intended radiation dose was 5 kGy, with the maximum limitation of no more than 10 kGy. Quality was controlled throughout this study, according to regulations, by the Secondary Standard Dosimetry Laboratory (SSDL, Thailand).

#### Sampling and enumeration

Twenty five grams of each herb was transferred into a sterile flask containing 225 ml sterile normal saline solution and let stand ~4 h at room temperature. After thorough mixing, 10-fold dilutions were performed and 0.1 ml was transferred onto appropriate agar medium and spreaded by a standard technique (FDA:BAM, 2001). All plates were incubated at 37°C for 24 h. Triplicate plates were prepared at each dilution from each herb sample, before and after gamma irradiation.

### Results and discussion

Before irradiation, levels of total aerobic bacteria ( $>10^5$ - $10^{11}$  CFU/g) (Table 2), *Staphylococcus* spp. ( $>10^3$ - $10^7$  CFU/g) (Table 3), *Salmonella* spp. ( $>10^4$ - $10^7$  CFU/g) (Table 4), coliform bacteria ( $>10^3$ - $10^7$  CFU/g) (Table 5), and fungi ( $>10^3$ - $10^6$  CFU/g) (Table 6) were quite high. None of these was detected in herb samples after ionizing radiation treatment. Only 7.7 and 8.8 kGy were used in this study; these are in the range of irradiation doses ( $>7.5$ - $10$  kGy) that have previously been shown to reduce microorganism totally or to 10 CFU/g in herb products (Tiengthavaj *et al.*, 1998). All irradiated herbs in this study were held in the same polyethylene bags at ambient temperature for 6 months and tested repeatedly. No microbial contamination was detected (data not shown). Moreover, examination by Thai herbs experts revealed no difference in aroma, color, or texture of the samples, before and after irradiation, of any of the herbs (data not shown). It is known that the sensory properties of most spices are well maintained between 7.5 and 15 kGy (WHO, 1994; FDA, 2001). Research clearly indicates that irradiation maintains the sensory properties of spices, herbs,

**Table 2. Total aerobic bacterial loads (CFU/g) in Thai medicinal herbs before irradiation (no viable counts were detected after irradiation).**

Scientific name	Trial number			
	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>b</sup>	4 <sup>b</sup>
1. <i>Aegle marmelos</i>	2.3×10 <sup>5</sup>	1.4×10 <sup>6</sup>	1.8×10 <sup>6</sup>	ND <sup>c</sup>
2. <i>Betula alnoides</i>	3.0×10 <sup>7</sup>	1.6×10 <sup>6</sup>	1.8×10 <sup>6</sup>	3.1×10 <sup>6</sup>
3. <i>Bixa orellana</i>	4.1×10 <sup>6</sup>	2.2×10 <sup>6</sup>	2.0×10 <sup>6</sup>	2.8×10 <sup>6</sup>
4. <i>Boesenbergia rotunda</i>	3.0×10 <sup>7</sup>	ND	ND	5.5×10 <sup>6</sup>
5. <i>Butea superba</i>	2.3×10 <sup>7</sup>	2.5×10 <sup>7</sup>	3.0×10 <sup>7</sup>	8.4×10 <sup>6</sup>
6. <i>Caesalpinia sappan</i>	1.4×10 <sup>7</sup>	1.5×10 <sup>6</sup>	1.4×10 <sup>6</sup>	1.2×10 <sup>6</sup>
7. <i>Cyperus rotundus</i>	3.0×10 <sup>7</sup>	3.0×10 <sup>7</sup>	1.8×10 <sup>7</sup>	2.9×10 <sup>6</sup>
8. <i>Ficus foveolata</i>	3.0×10 <sup>5</sup>	1.1×10 <sup>6</sup>	2.0×10 <sup>6</sup>	1.1×10 <sup>6</sup>
9. <i>Mesua ferrea</i>	2.8×10 <sup>6</sup>	1.3×10 <sup>6</sup>	1.8×10 <sup>6</sup>	7.5×10 <sup>5</sup>
10. <i>Mimusops elengi</i>	3.6×10 <sup>6</sup>	1.9×10 <sup>6</sup>	1.6×10 <sup>6</sup>	9.4×10 <sup>5</sup>
11. <i>Mucuna collettii</i>	1.4×10 <sup>10</sup>	1.9×10 <sup>6</sup>	1.7×10 <sup>6</sup>	1.4×10 <sup>6</sup>
12. <i>Phyllanthus emblica</i>	2.0×10 <sup>11</sup>	2.2×10 <sup>6</sup>	1.8×10 <sup>6</sup>	2.9×10 <sup>6</sup>
13. <i>Piper retrofractum</i>	1.4×10 <sup>7</sup>	1.8×10 <sup>6</sup>	2.0×10 <sup>6</sup>	8.9×10 <sup>5</sup>
14. <i>Pueraria mirifica</i>	3.2×10 <sup>10</sup>	2.2×10 <sup>7</sup>	2.3×10 <sup>7</sup>	9.2×10 <sup>6</sup>
15. <i>Terminalia belerica</i>	1.0×10 <sup>7</sup>	2.0×10 <sup>6</sup>	1.5×10 <sup>6</sup>	1.1×10 <sup>5</sup>
16. <i>Terminalia chebula</i>	1.1×10 <sup>7</sup>	1.9×10 <sup>6</sup>	2.3×10 <sup>6</sup>	4.6×10 <sup>6</sup>
17. <i>Tinospora crispa</i>	1.3×10 <sup>9</sup>	1.8×10 <sup>6</sup>	1.8×10 <sup>6</sup>	1.8×10 <sup>6</sup>

<sup>a</sup> irradiation dose = 7.7 kGy <sup>b</sup> irradiation dose = 8.8 kGy <sup>c</sup> ND = not done

**Table 3. *Staphylococcus* spp. Loads (CFU/g) in Thai medicinal herbs before irradiation (no viable counts were detected after irradiation).**

Scientific name	Trial number			
	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>b</sup>	4 <sup>b</sup>
1. <i>Aegle marmelos</i>	3.3×10 <sup>4</sup>	- <sup>c</sup>	1.9×10 <sup>5</sup>	ND <sup>d</sup>
2. <i>Betula alnoides</i>	3.9×10 <sup>4</sup>	-	1.0×10 <sup>6</sup>	1.0×10 <sup>6</sup>
3. <i>Bixa orellana</i>	1.2×10 <sup>6</sup>	-	-	2.5×10 <sup>4</sup>
4. <i>Boesenbergia rotunda</i>	3.0×10 <sup>7</sup>	ND	ND	2.4×10 <sup>5</sup>
5. <i>Butea superba</i>	8.9×10 <sup>4</sup>	6.7×10 <sup>5</sup>	1.3×10 <sup>4</sup>	1.0×10 <sup>5</sup>
6. <i>Caesalpinia sappan</i>	-	-	-	1.1×10 <sup>5</sup>
7. <i>Cyperus rotundus</i>	5.2×10 <sup>4</sup>	-	-	2.7×10 <sup>4</sup>
8. <i>Ficus foveolata</i>	1.1×10 <sup>5</sup>	-	-	1.4×10 <sup>4</sup>
9. <i>Mesua ferrea</i>	5.1×10 <sup>4</sup>	-	8.0×10 <sup>3</sup>	2.0×10 <sup>5</sup>
10. <i>Mimusops elengi</i>	8.0×10 <sup>3</sup>	-	7.7×10 <sup>3</sup>	1.2×10 <sup>5</sup>
11. <i>Mucuna collettii</i>	3.2×10 <sup>4</sup>	1.2×10 <sup>6</sup>	3.4×10 <sup>5</sup>	2.3×10 <sup>5</sup>
12. <i>Phyllanthus emblica</i>	1.7×10 <sup>4</sup>	3.2×10 <sup>5</sup>	6.4×10 <sup>4</sup>	3.2×10 <sup>4</sup>
13. <i>Piper retrofractum</i>	3.0×10 <sup>3</sup>	-	-	1.6×10 <sup>4</sup>
14. <i>Pueraria mirifica</i>	3.2×10 <sup>4</sup>	1.2×10 <sup>6</sup>	-	4.2×10 <sup>5</sup>
15. <i>Terminalia belerica</i>	2.0×10 <sup>3</sup>	1.2×10 <sup>6</sup>	-	1.0×10 <sup>4</sup>
16. <i>Terminalia chebula</i>	5.1×10 <sup>4</sup>	-	-	1.6×10 <sup>4</sup>
17. <i>Tinospora crispa</i>	3.8×10 <sup>5</sup>	1.3×10 <sup>6</sup>	1.0×10 <sup>5</sup>	2.0×10 <sup>5</sup>

<sup>a</sup> irradiation dose = 7.7 kGy <sup>b</sup> irradiation dose = 8.8 kGy <sup>c</sup> - = no viable counts detected

<sup>d</sup> ND = not done

**Table 4. *Salmonella* spp. Loads (CFU/g) in Thai medicinal herbs before irradiation (no viable counts were detected after irradiation).**

Scientific name	Trial number			
	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>b</sup>	4 <sup>b</sup>
1. <i>Aegle marmelos</i>	8.0×10 <sup>5</sup>	- <sup>c</sup>	9.1×10 <sup>5</sup>	ND <sup>d</sup>
2. <i>Betula alnoides</i>	-	-	1.4×10 <sup>6</sup>	-
3. <i>Bixa orellana</i>	-	4.9×10 <sup>4</sup>	-	2.1×10 <sup>5</sup>
4. <i>Boesenbergia rotunda</i>	-	ND	ND	-
5. <i>Butea superba</i>	3.1×10 <sup>4</sup>	-	-	-
6. <i>Caesalpinia sappan</i>	5.4×10 <sup>4</sup>	-	-	4.4×10 <sup>5</sup>
7. <i>Cyperus rotundus</i>	-	1.4×10 <sup>5</sup>	-	1.5×10 <sup>5</sup>
8. <i>Ficus foveolata</i>	-	-	1.4×10 <sup>6</sup>	-
9. <i>Mesua ferrea</i>	4.0×10 <sup>5</sup>	-	-	1.7×10 <sup>5</sup>
10. <i>Mimusops elengi</i>	-	1.6×10 <sup>5</sup>	-	7.6×10 <sup>4</sup>
11. <i>Mucuna collettii</i>	-	9.6×10 <sup>5</sup>	8.6×10 <sup>5</sup>	2.9×10 <sup>6</sup>
12. <i>Phyllanthus emblica</i>	-	2.4×10 <sup>5</sup>	-	1.4×10 <sup>5</sup>
13. <i>Piper retrofractum</i>	-	8.0×10 <sup>4</sup>	1.7×10 <sup>6</sup>	3.3×10 <sup>5</sup>
14. <i>Pueraria mirifica</i>	-	9.1×10 <sup>5</sup>	7.8×10 <sup>5</sup>	9.1×10 <sup>5</sup>
15. <i>Terminalia belerica</i>	-	-	-	-
16. <i>Terminalia chebula</i>	-	1.7×10 <sup>6</sup>	-	-
17. <i>Tinospora crispa</i>	3.0×10 <sup>7</sup>	6.6×10 <sup>5</sup>	1.4×10 <sup>6</sup>	-

<sup>a</sup> irradiation dose = 7.7 kGy <sup>b</sup> irradiation dose = 8.8 kGy <sup>c</sup> - = no viable counts detected

<sup>d</sup> ND = not done

**Table 5. Coliform bacterial loads (CFU/g) in Thai medicinal herbs before irradiation (no viable counts were detected after irradiation).**

Scientific name	Trial number			
	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>b</sup>	4 <sup>b</sup>
1. <i>Aegle marmelos</i>	- <sup>c</sup>	9.6×10 <sup>5</sup>	-	ND <sup>d</sup>
2. <i>Betula alnoides</i>	-	6.7×10 <sup>5</sup>	-	2.0×10 <sup>6</sup>
3. <i>Bixa orellana</i>	-	1.5×10 <sup>6</sup>	-	1.2×10 <sup>6</sup>
4. <i>Boesenbergia rotunda</i>	-	ND	ND	2.1×10 <sup>6</sup>
5. <i>Butea superba</i>	1.6×10 <sup>7</sup>	1.1×10 <sup>6</sup>	9.1×10 <sup>5</sup>	1.0×10 <sup>6</sup>
6. <i>Caesalpinia sappan</i>	-	-	-	5.4×10 <sup>5</sup>
7. <i>Cyperus rotundus</i>	1.0×10 <sup>3</sup>	1.5×10 <sup>6</sup>	-	1.4×10 <sup>6</sup>
8. <i>Ficus foveolata</i>	-	-	-	4.1×10 <sup>6</sup>
9. <i>Mesua ferrea</i>	-	-	-	3.2×10 <sup>5</sup>
10. <i>Mimusops elengi</i>	2.0×10 <sup>3</sup>	1.1×10 <sup>6</sup>	-	3.7×10 <sup>5</sup>
11. <i>Mucuna collettii</i>	-	1.3×10 <sup>5</sup>	2.4×10 <sup>5</sup>	-
12. <i>Phyllanthus emblica</i>	-	7.2×10 <sup>5</sup>	4.8×10 <sup>4</sup>	3.6×10 <sup>5</sup>
13. <i>Piper retrofractum</i>	-	1.4×10 <sup>6</sup>	-	1.8×10 <sup>5</sup>
14. <i>Pueraria mirifica</i>	7.5×10 <sup>4</sup>	1.1×10 <sup>6</sup>	1.1×10 <sup>6</sup>	2.6×10 <sup>6</sup>
15. <i>Terminalia belerica</i>	-	-	-	2.8×10 <sup>6</sup>
16. <i>Terminalia chebula</i>	-	-	-	6.1×10 <sup>6</sup>
17. <i>Tinospora crispa</i>	3.7×10 <sup>7</sup>	-	-	1.4×10 <sup>6</sup>

<sup>a</sup> irradiation dose = 7.7 kGy <sup>b</sup> irradiation dose = 8.8 kGy <sup>c</sup> - = no viable counts detected

<sup>d</sup> ND = not done

**Table 6. Fungi (CFU/g) in Thai medicinal herbs before irradiation (no viable counts were detected after irradiation).**

Scientific name	Trial number			
	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>b</sup>	4 <sup>b</sup>
1. <i>Aegle marmelos</i>	- <sup>c</sup>	8.0×10 <sup>4</sup>	2.2×10 <sup>5</sup>	ND <sup>d</sup>
2. <i>Betula alnoides</i>	-	-	-	2.6×10 <sup>3</sup>
3. <i>Bixa orellana</i>	-	-	-	5.6×10 <sup>4</sup>
4. <i>Boesenbergia rotunda</i>	-	ND	ND	6.4×10 <sup>4</sup>
5. <i>Butea superba</i>	-	2.9×10 <sup>5</sup>	1.9×10 <sup>6</sup>	8.0×10 <sup>4</sup>
6. <i>Caesalpinia sappan</i>	-	2.4×10 <sup>5</sup>	1.6×10 <sup>4</sup>	-
7. <i>Cyperus rotundus</i>	5.0×10 <sup>3</sup>	-	1.0×10 <sup>3</sup>	4.8×10 <sup>4</sup>
8. <i>Ficus foveolata</i>	1.0×10 <sup>3</sup>	-	8.0×10 <sup>3</sup>	-
9. <i>Mesua ferrea</i>	1.1×10 <sup>4</sup>	-	-	-
10. <i>Mimusops elengi</i>	-	-	1.1×10 <sup>3</sup>	3.2×10 <sup>3</sup>
11. <i>Mucuna collettii</i>	-	1.6×10 <sup>4</sup>	2.0×10 <sup>5</sup>	-
12. <i>Phyllanthus emblica</i>	-	-	-	-
13. <i>Piper retrofractum</i>	-	9.6×10 <sup>4</sup>	3.2×10 <sup>4</sup>	5.3×10 <sup>3</sup>
14. <i>Pueraria mirifica</i>	-	4.0×10 <sup>5</sup>	3.4×10 <sup>5</sup>	5.2×10 <sup>5</sup>
15. <i>Terminalia belerica</i>	-	-	5.6×10 <sup>4</sup>	-
16. <i>Terminalia chebula</i>	-	-	4.0×10 <sup>4</sup>	-
17. <i>Tinospora crispa</i>	4.7×10 <sup>4</sup>	8.0×10 <sup>4</sup>	1.5×10 <sup>5</sup>	2.4×10 <sup>3</sup>

<sup>a</sup> irradiation dose = 7.7 kGy <sup>b</sup> irradiation dose = 8.8 kGy <sup>c</sup> - = no viable counts detected

<sup>d</sup> ND = not done

and vegetable seasonings better than ethylene dioxide treatment (Food and Environmental Protection Section Joint FAO/IAEA Division Nuclear Techniques in Food and Agriculture International Atomic Energy Agency, 1999).

According to microbiological criteria for spices that have been recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1974), spices are of an unacceptable quality when the bacterial counts exceed 10<sup>6</sup> CFU/g and the numbers of molds are higher than 10<sup>4</sup> CFU/g. This standard was applied in the present study, since no specification has been adopted for microorganism counts in herbs. All year round post-harvested and powdered herbs in this study were contaminated with microorganisms, as shown by total bacterial or aerobic mesophilic bacteria counts in the range of 10<sup>5</sup>-10<sup>11</sup> CFU/g and mold counts in the range of 10<sup>3</sup>-10<sup>5</sup> CFU/g. The microbial contamination exceeded acceptable levels for human use in many samples.

*Staphylococcus* spp. seemed to be the major bacteria in powdered herbs in all seasons, especially in the herbs harvested in the rainy and summer seasons. *Salmonella* spp. were detected mostly in certain herbs, which were harvested in summertime. Some species of *Staphylococcus* and all serotypes of *Salmonella* spp. are foodborne pathogens for humans, which is a serious concern (Meng and Doyle, 1998; Leclerc *et al.*, 2002; McCabe-Sellers and Beattie, 2004; Lin *et al.*, 2005). The herbs could be contaminated by human handlers during harvesting or processing. Coliform bacteria were also detected in all herbs, at levels varying with the season. The highest levels were found in herbs harvested in the summer. High temperatures in the summer season apparently enhanced bacterial growth, even in dried-powdered herbs. The presence of coliforms implied the possibility of fecal contamination and inadequate sanitation management while the herbs were being grown. Meanwhile the total fungi counts were in the range

of  $\sim 10^3$ - $10^6$  CFU/g; the majority were yeasts, which are reported to be common flora on plant surfaces, especially on fruits.

Herbs are naturally contaminated with high numbers of bacteria and fungi, which may be normal flora *in situ*; the flora differs depending on what parts of the plant harvested for use (Frazier and Westhoff, 1978). Some Thai herbs were often collected from wild areas that may be highly contaminated by the feces of wild animals. Avoiding high levels of microbial contamination in herbs may be impossible. Herbs are relatively sensitive to treatment of any kind and are particularly damaged by ethylene dioxide treatment. Irradiation is an effective technology for resolving technical trade issues for many food and agricultural products (WHO, 1994; FDA, 2001). As a disinfection treatment, it offers the possibility of targeting different levels of quarantine security and is one of few methods able to control internal pests (FDA, 2001). As a disinfection treatment, it offers good broad-spectrum control of many pathogenic and spoilage organisms with minimal change to the herbs. As well as the price since only 8 baht per kg of herb is charged by Thai Irradiation Center, Office of Atoms for Peace, Ministry of Science and Technology, Klong 5, Klonglaung, Pathum Thani, Thailand. Thus, gamma irradiation at doses of  $>7.5$ - $10$  kGy is definitely safe for the treatment of microbial contamination in Thai main medicinal herbs before they are manufactured into a variety of traditional medicines and food supplement.

### Acknowledgements

The authors sincerely thank Professor Dean Cliver, University of California, Davis, for revising the manuscript and providing many valuable suggestions and comments. This research was supported by the Thailand Research Fund.

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