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Effect of Different Decontamination Methods on Microbiological Aspects, Bioactive Constituents and Antibacterial Activity of Turmeric (*Curcuma longa* Linn.) Powder

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Authors' contributions

SC, SS, and SV supervised in the design of the study and contributed to the writing process. NT, CB, CC, and JC carried out the experiments, performed the statistical analysis, and wrote the protocol. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Turmeric (*Curcuma longa* Linn.) has long been widely used for food, food additives, and traditional medicine. This study was aimed to assess effects of different decontamination procedures of turmeric rhizomes on microbiological quality and bioactive constituents of aseptically prepared turmeric powder. In addition, antibacterial activity of the powder on skin and wound pathogens was performed. The tested rhizomes were decontaminated by different methods including soaking in 70% (v/v) ethanol for 15 min, boiling in water for 15 min, boiling in 5% (v/v) acetic acid for 15 min, steaming at 100°C for 15 min and autoclaving at 121°C and 15 psi for 15 min. There were no foreign materials detected among the tested samples. The moisture content of each analyzed powder was similar (6.1±0.4%; v/w). The microbial contamination of the turmeric samples prepared from the ethanol soaked rhizome, water boiled rhizome, acetic acid boiled rhizome, and autoclaved

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rhizome were of satisfactory quality as required by the Thai Herbal Pharmacopoeia (THP) standard. Even though, the decontamination processes altered the contents of ethanol- and water-soluble extractives, curcuminoids, and volatile oils of the turmeric products but all of them conform to the THP standard. Among these techniques, the autoclave method was found to be sufficient for complete microbial decontamination without significantly affecting on the active constituents of the turmeric powder.

Keywords: Antibacterial activity; *Curcuma longa* Linn; food safety; microbial contamination; medicinal plant; turmeric powder.

1. INTRODUCTION

Curcuma longa Linn. (Zingiberaceae) commonly known as turmeric is traditionally used as a spice and coloring agent in Asian cuisine. Turmeric possesses a wide range of biological activities such as anti-microbial, anti-cancer, anti-inflammatory and anti-oxidant activities (Aggarwal et al., 2003; Houssem et al., 2010; Rivera-Espinoza and Muriel, 2009). Scientific information suggested a logical basis for its traditional utilization in healthy food. At the present day, the spice is extensively employed in pharmaceutical and cosmetic industries as an effective mean for the treatment of various skin diseases and liver disorders. It is also used for its carminative, stomachic and laxative properties (Kim et al., 2011; Thangapazham et al., 2007; Zhao et al., 2011). Curcuminoids consisting of curcumin (50-60%), demethoxy curcumin (20-30%) and bis-demethoxy curcumin (7-20%) and volatile oils consisting of - phellandrene, 1:8 cineol, zingiberene, ar-curcumene, turmerone, ar-turmerone, - sesquiphellandrene, curlone and dehydrozingerone have been well-documented as the major active constituents of turmeric (Manzan et al., 2003).

Microbiological contamination of herbs and spices may provide a possible conduit to introduce food spoilage organisms into a wide range of dishes. In addition, spices and herbs may contain pathogens that cause a risk to public health, particularly *Salmonella* spp., *Escherichia coli* and endospore-forming and toxin-producers such as *Clostridium perfringens*. Yeasts and moulds have generally been found in herbs and spices and may create serious problems due to aflatoxin production (Kosalec et al., 2009). Microbial decontamination by ethylene oxide or methyl bromide is prohibited in several countries, for health, environment, and occupational safety reasons (Sharma et al., 2010). Decontamination achieved by gamma radiation is one of the currently practiced methods of reducing microbial load and disinfecting food and spices (Rivera et al., 2010; Saxena et al., 2010; Sharma et al., 2010). The use of ionizing radiation is accepted in many countries and recommended by a number of organizations, but only well-trained and experienced staff should decide upon the desirability of its use. Moreover, specially designed installations and equipment must be used; therefore, this decontamination method is not suitable as an in-house process (Mohammad et al., 2009).

This present investigation was aimed to evaluate the effect of several simple low cost methods to decontaminate turmeric rhizomes on microbiological quality and bioactive constituents of aseptically prepared turmeric powder. In addition, antibacterial activity of the powder on selected important skin and wound pathogens such as *Staphylococcus* spp., *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa* was performed. This information should be of interest to manufacturers and consumers in order to obtain an easy, inexpensive decontamination method for household applications.

2. MATERIALS AND METHODS

2.1 Preparation of Turmeric Powder

Fresh rhizomes (bulbs) of turmeric (10-month old) were purchased from Ban Ta Khun, Suratthani, Thailand. The rhizomes (500 g each) were washed with water and subjected to decontamination processes by (i) soaking in five liters of 70% ethanol for 15 min, (ii) boiling in five liters of water at 100°C for 15 min, (iii) boiling in five liters of 5% (v/v) acetic acid: water at 100°C for 15 min, (iv) steaming at 100°C for 15 min and (v) autoclaving at 121°C and 15 psi for 15 min. A clean non-decontaminated rhizome was included as control.

Each sample of turmeric rhizomes was aseptically sliced into small pieces, approximately 0.5 cm in thickness and dried in an oven at 50°C for 48 h. The rhizomes were then macerated into fine powder and passed through a sieve No. 40 (425 µm). The turmeric powder from different decontaminated rhizomes was separately packed in sterile black polyethylene bags and stored at 25°C (Subhadhirasakul et al., 2007).

All experimental procedures unless otherwise stated were carried out according to Thai Herbal Pharmacopoeia (THP), Department of Medical Sciences, Bangkok, Thailand (Thai Herbal Pharmacopoeia, 2009). Independently three subsamples were taken for verification their quality by determination of foreign matter, moisture contents, microbiological aspects, and bioactive principles. All experiments were replicated three times with each sample. Microbial analyses and bioactive principles were initiated within 24 h after the powder preparation.

Each sample was verified for its quality by determination of foreign matter and moisture contents. The powder (100 g) was spread in a thin layer and foreign matters were separated into groups by visual inspection, using a 6X magnifying lens. The sorted foreign matter was weighed and content calculated in dried samples (%; w/w). Besides, 50g of the turmeric powder was accurately weighed and quantitated for moisture content by azeotropic distillation method.

2.2 Microbiological Aspects of Turmeric Powder

Ten gram of turmeric powder was aseptically weighed into a sterile 100 mL-volumetric flask, and the expected volume was adjusted with soybean-casein digest broth (Oxoid, Basingstoke, UK). Total aerobic microbial count was determined on plate count agar (Difco, Maryland, USA) by pour-plating method followed by incubation at 35°C for 48 h. The number of colonies was counted and the average number of microorganisms per gram of each sample was then calculated. The number of bile-tolerant Gram negative bacteria was specified by pour plating into molten MacConkey agar (Difco, Detroit, USA) after incubation at 35°C for 24-48 h. Total combined yeasts and moulds were assessed on pour-plates of Sabouraud dextrose agar (Difco, Detroit, USA) followed by incubation at room temperature for 5-7 days.

Moreover, ten gram of turmeric powder was preincubated into soybean-casein digest broth (1:10; w/v) at 35°C for 24 h. The enrichment culture was employed for testing the presence of some important pathogens. *S. aureus* were distinguished by spread-plating on mannitol salt agar (Oxoid, Basingstoke, UK) after incubation at 37°C for 24 h. Colonies with suggestive morphology of staphylococci were submitted to Gram staining, catalase and

coagulase tests. *P. aeruginosa* was detected on spread-plates of cetrimide agar (Difco, MD, USA) after incubation at 35°C for 24 h. The green colonies were tested for oxidase reaction and subcultured into Triple sugar iron medium. Growth of bacteria and the reaction results were observed. The presence of *E. coli* was enumerated by spread-plating on MacConkey agar and presumptive *E. coli* colonies were further tested using eosin methylene blue agar (Difco, Detroit, USA). After 24 h incubation at 35°C, Gram staining of typical colonies of *E. coli* was conducted. The enrichment culture was separately transferred into bismuth sulphite agar, brilliant green agar, and Salmonella Shigella agar (Oxoid, Basingstoke, UK). The presence of typical *Salmonella* spp. colonies was recorded after 24 h incubation at 35°C. *Clostridium* spp. were analyzed by adding the culture into cooked meat medium (Difco, Detroit, USA). The culture was subsequently streaked on tryptose sulphite cycloserine egg yolk agar (Merck KGaA, Darmstadt, Germany) and incubated at 35°C for 24 h under anaerobic condition. All experiments were replicated three times with different turmeric samples. Duplicate samples were analyzed per replicate.

2.3 Determination of Bioactive Principles of Turmeric Powder

Ethanol- and water-soluble extractives in the samples were performed in accordance with the procedure described previously (Thai Herbal Pharmacopoeia 2009). Five gram of turmeric powder was accurately weighed and macerated with 100 mL of 95% ethanol (Lab Scan, Dublin, Ireland) for 24 h. The solvent was filtered and dried at 105°C to constant weight. The percentage of ethanol-soluble extract was calculated as the solvent extractive in the samples (%; w/w). The procedure followed as above using chloroform water (Merck KGaA, Darmstadt, Germany) instead of ethanol was carried out to obtain the percentage of water-soluble extractive.

Quantitative analysis of total curcuminoids in the samples was determined by UV-spectrophotometer (Model Genesis 5, Miltonroy, USA.). Standard curcumin solutions were prepared by using standard curcumin (Fluka, Switzerland) in methanol to obtain about 0.78, 1.56, 1.95, 2.34 and 3.12µg/ml (Thai Herbal Pharmacopoeia, 2009). The absorption intensities of the standard and sample solutions were measured at 420 nm and methanol was used as a blank. The concentration of curcuminoids in the test samples was calculated from the calibration curve. The percentage of total curcuminoids was expressed as the weight of total curcuminoids per 100g dried weight of stored sample.

Volatile oil content was determined by a Dean stark apparatus according to Thai Herbal Pharmacopoeia (THP), Department of Medical Sciences, Bangkok, Thailand (Thai Herbal Pharmacopoeia, 2009). The volatile oil content was calculated and expressed as a volume of the oil per 100g of the sample (% v/w calculated on dried basis).

All experiments were replicated three times with different turmeric samples. Triplicate samples were analyzed per replicate. Results are expressed as mean ± SEM (standard error of the mean) of "n" samples. Statistical significance between controls and treatments was determined using the Student's *t*-test.

2.4 Antibacterial Activity of the Turmeric Powder

Foodborne pathogens and skin and wound pathogens provided by Natural Product Research Center, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand were

used in this study. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Acinetobacter baumannii* ATCC 19606 were included as quality control strains.

Antibacterial activity of the turmeric powder against the pathogens was conducted by disc diffusion method (CLSI 2009). An aliquot of 10 μ L of 250mg/mL of each extract was individually applied to sterile filter paper discs (Whatman no. 1; 6 mm in diameter). The discs were placed on the surface of Mueller Hinton agar (Difco, MD, USA) plates that previously seeded with the culture of the tested bacteria (10⁸ CFU/mL). Antibiotic susceptibility discs including penicillin G (10U/disc) and ciprofloxacin (5 μ g/disc) were used as controls while 10 μ L of dimethyl sulfoxide (Merck KGaA, Darmstadt, Germany) was included as negative control. The plates were then incubated for 24 h at 37°C. The antibacterial activity was evaluated by measuring the diameter of inhibition zones.

3. RESULTS

The turmeric powder from the different decontaminated rhizomes was evaluated for several parameters according to THP. There was no foreign material detected in the analyzed powder (data not shown). The moisture content of the samples was lower than the THP requirement (ranging from 5.8-6.8%; v/w) and it was not statistically different among the test powder (Fig. 1). Applying the criteria in the THP, four powders from the different decontaminated turmeric rhizomes were of satisfactory microbiological quality (Table 1). The batches of turmeric powder from clean non-sterile rhizomes and the steamed rhizomes were of insufficient quality due to the presence of *Salmonella* spp., and/or high level of total aerobic microbial count, total combined yeasts and molds, and bile-tolerant Gram negative bacteria ranging from 1.3x10⁶, 0.4-1.2x10⁴ and 0.4-1.3x10⁴ CFU/g, respectively.

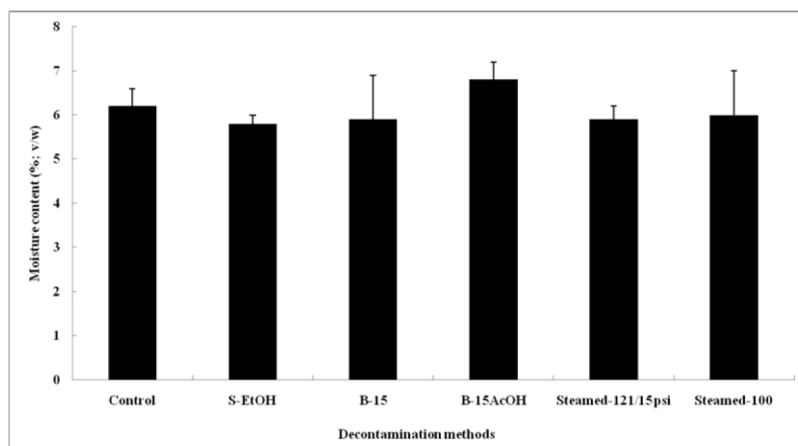


Fig. 1. Moisture content of turmeric powder prepared from different decontaminated *Curcuma longa* rhizome including, soaking in 70% ethanol for 15 min (S-EtOH), boiling for 15 min (B-15), boiling in 5% acetic acid for 15 min (B-15AcOH), steaming at 100°C for 15 min (Steamed-100) and autoclaving at 121°C 15 psi for 15 min (Steamed-121/15 psi).

Table 1. Effects of different decontaminated turmeric rhizomes on the microbial count in turmeric powder. The decontamination procedures were soaking in 70% ethanol for 15 min (S-EtOH), boiling for 15 min (B-15), boiling in 5% acetic acid for 15 min (B-15AcOH), steaming at 100 °C for 15 min (Steamed-100), and autoclaving at 121 °C and 15 psi for 15 min (Steamed-121/15 psi) on the microbial count in turmeric powder.

Tested microbial	Viable count (CFU/g)					
	Control	S-EtOH	B-15	B-15AcOH	Steamed-121/15 psi	Steamed-100
Total aerobic microbial count	$(1.3 \pm 0.6) \times 10^6$	ND ¹	ND	ND	ND	$(2.3 \pm 0.7) \times 10^3$
Total combined yeasts and moulds	$(1.4 \pm 0.1) \times 10^4$	ND	ND	ND	ND	$(3.8 \pm 0.9) \times 10^3$
Bile-tolerant Gram negative bacteria	$(1.3 \pm 0.3) \times 10^4$	ND	ND	ND	ND	$(4.0 \pm 0.2) \times 10^3$
<i>Staphylococcus aureus</i>	ND	ND	ND	ND	ND	ND
<i>Escherichia coli</i>	ND	ND	ND	ND	ND	ND
<i>Pseudomonas aeruginosa</i>	ND	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	P ²	ND	ND	ND	ND	P
<i>Clostridium</i> spp.	ND	ND	ND	ND	ND	ND

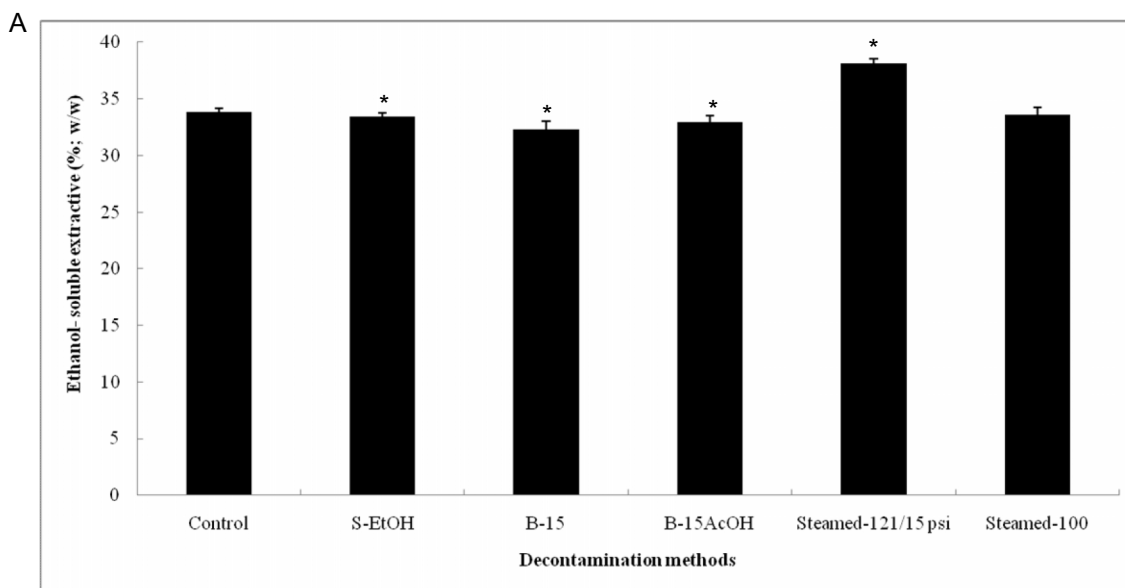
¹ ND; Not Detected

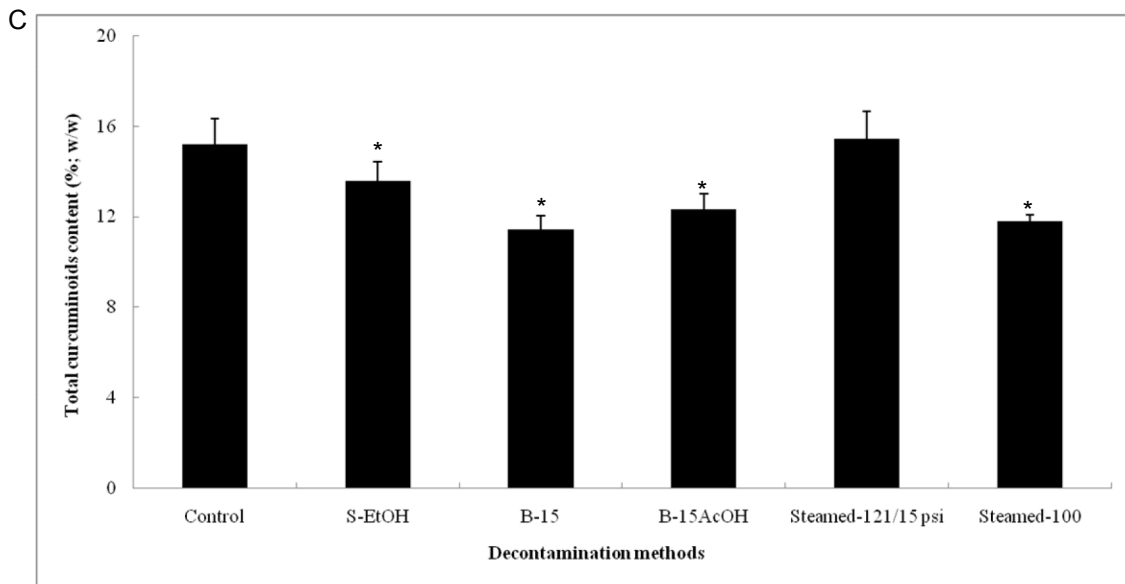
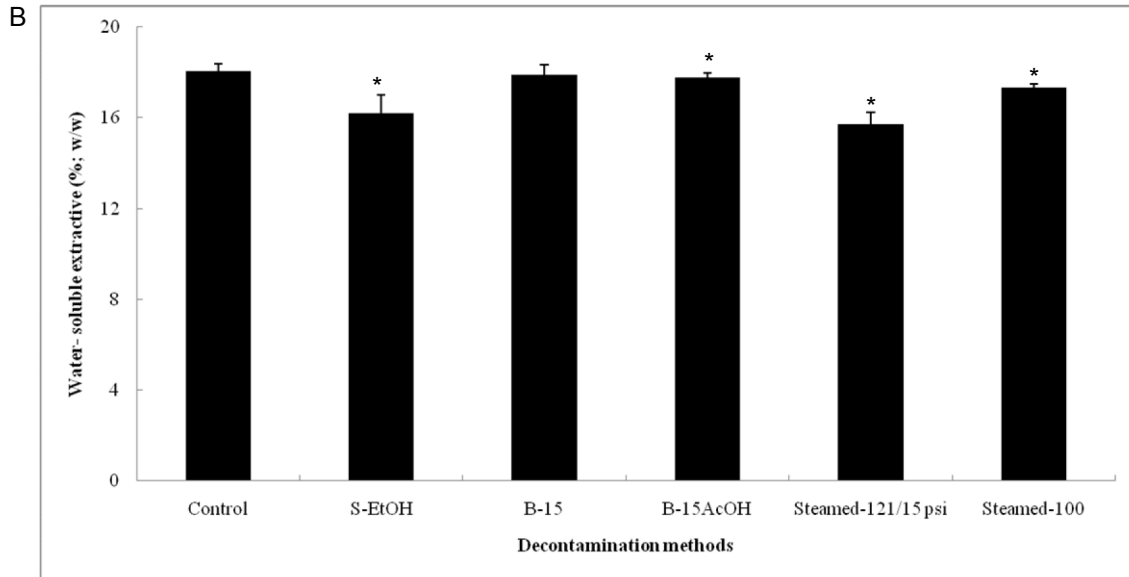
² P; Presence of the tested microorganisms

The extractive values of each sample that depicted in Figs. 2A and 2B were recorded in ethanol and water for studying the distribution of its constituents. The results show that active ingredients of the turmeric powder were more soluble in ethanol than water with the average of extractive values of 34.0 ± 2.1 and $17.2 \pm 1.0\%$ (w/w), respectively. The extractive values were found to be largely higher than the requirement in the THP. The maximum ethanol-soluble extractive yield was obtained from the sample prepared from the high pressure steamed rhizome, while the highest water-soluble extractive yield was got from the powder of the control rhizome.

As illustrated in Fig. 2C, total curcuminoid content of the analyzed turmeric samples ranged from 11.4 to 15.4% (w/w) which was within the limits as given in the THP. It should be noted that, curcuminoid contents of the powder were two to three times higher than the amount recommended by the THP. The powder of the high pressure steamed rhizome contained the highest content, while the sample of the water boiled rhizome was the lowest.

Fig. 2D gives the percent yield of volatile oils obtained from the decontaminated rhizome powder and the control rhizome powder. No significant differences of volatile oils content could be observed in the sample, $15.7 \pm 0.1\%$ (v/w). The contents were 2.5-times higher than a standard content of the THP. The slightly high volatile oils content was found in the powder from the high pressure steamed rhizome.





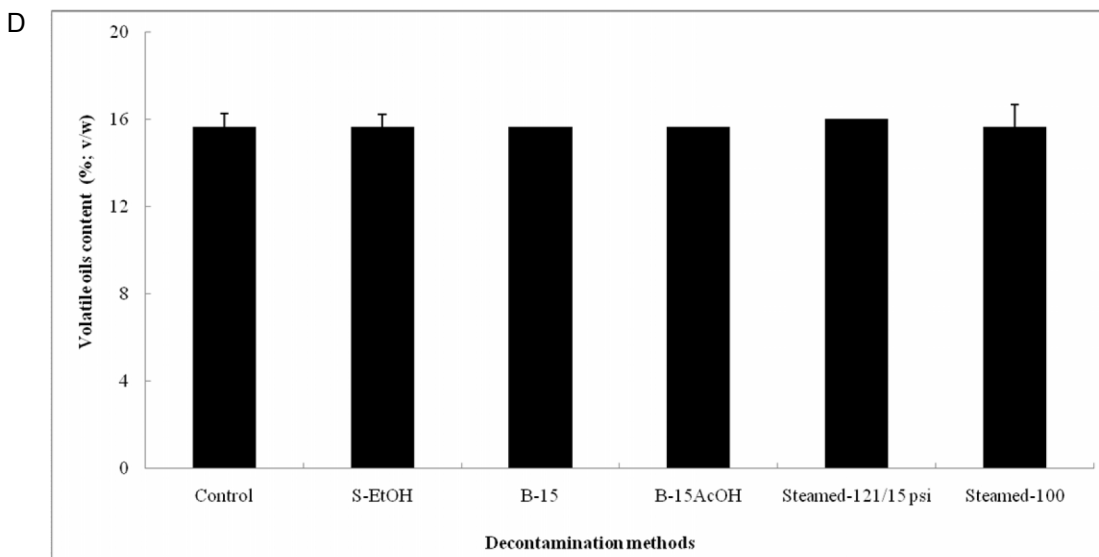


Fig. 2. Effects of decontamination methods for turmeric rhizomes on the bioactive principles detected in the powder. The decontamination procedures for the rhizomes were soaking in 70% ethanol for 15 min (S-EtOH), boiling for 15 min (B-15), boiling in 5% acetic acid for 15 min (B-15AcOH), steaming at 100°C for 15 min (Steamed-100), and autoclaving at 121°C 15 psi for 15 min (Steamed-121/15 psi). Ethanol-soluble extractive (A), water-soluble extractive (B), curcuminoid contents (C), and volatile oils contents (D) were the active constituents detected in turmeric powder. Non-decontaminated raw material was included as control.

*** Mean values are significantly different from control ($P < 0.05$).**

Antibacterial activity of each turmeric powder against both foodborne pathogens and skin and wound pathogens were compared as presented in Table 2. All tested turmeric powder at 2.5 mg/disc exhibited inhibitory effects against only Gram positive pathogens, including *B. cereus* TISTR 2647, MRSA, MSSA, *S. epidermidis*, coagulase positive staphylococci, and coagulase negative staphylococci. The mean values of the inhibition zones ranged from 7.0 to 11.5 mm.

Table 2. Antibacterial activity of turmeric powder prepared from different decontaminated rhizomes. The decontamination procedures were soaking in 70% ethanol for 15 min (S-EtOH), boiling for 15 min (B-15), boiling in 5% acetic acid for 15 min (B-15AcOH), steaming at 100°C for 15 min (Steamed-100), and autoclaving at 121°C and 15 psi for 15 min (Steamed-121/15 psi)

Tested bacterial strains	Inhibition zones of different turmeric powder (2.5 mg/disc)/antibiotics ¹ (Mean values ± SEs; mm)						
	Control	S-EtOH	B-15	B-15AcOH	Steamed-121/15 psi	Steamed-100	Antibiotics
MRSA NPRC R001	8.7±0.7	8.9±0.9	8.5±0.2	9.9±0.9	7.9±0.4	8.3±0.6	-
MSSA NPRC S001	8.3±1.0	7.7±0.1	10.1±1.0	10.3±0.4	8.2±1.0	7.9±0.2	21.0±0.3
CPS NPRC 301	7.9±0.2	8.2±0.3	8.0±0.0	8.6±0.3	7.8±0.1	7.2±0.0	24.1±0.1
CNS NPRC 501	9.7±0.1	8.0±0.4	8.5±0.1	11.5±0.7	8.2±0.3	8.2±0.3	13.3±0.3
<i>B. cereus</i> TISTR 2647	5.7±0.1	5.6±0.1	ND ³	6.5±0.2	5.6±0.1	5.8±0.2	ND
<i>L. monocytogenes</i>	- ²	-	ND	-	-	-	ND
<i>S. aureus</i> ATCC 25923	9.9±0.3	9.5±0.2	8.6±0.1	9.7±0.1	9.4±0.5	8.5±0.4	24.0±0.3
<i>S. aureus</i> NPRU 401	7.0±0.8	7.1±0.1	ND	8.6±0.7	7.8±0.6	7.9±0.1	ND
<i>S. aureus</i> NPRU 411	-	-	ND	-	-	-	ND
<i>S. epidermidis</i> ATCC 35984	8.8±0.3	9.1±0.0	9.00±0.0	9.2±0.1	8.5±0.2	8.8±0.3	-
<i>S. epidermidis</i> ATCC 122228	8.3±0.4	8.9±0.5	8.2±0.0	10.6±0.1	8.2±0.3	8.2±0.3	10.0±0.3
MDR <i>A. baumannii</i> JVC 105	-	-	-	-	-	-	-
MDR <i>A. baumannii</i> NPRC AB013	-	-	-	-	-	-	-
MDR <i>P. aeruginosa</i> NPRC PA001	-	-	-	-	-	-	-
MDR <i>E. coli</i> NPRC EC05	-	-	-	-	-	-	8.7±0.3
<i>A. baumannii</i> ATCC19606	-	-	-	-	-	-	23.3±0.2
<i>E. coli</i> ATCC 25922	-	-	-	-	-	-	16.0±0.1
<i>E. coli</i> O157: H7	-	-	ND	-	-	-	ND
<i>S. Typhi</i>	-	-	ND	-	-	-	ND
<i>V. parahaemolyticus</i>	-	-	ND	-	-	-	ND

¹; The antibiotics including, penicillin G (10 U/disc) and ciprofloxacin (5 µg/disc) were applied for Gram positive and Gram negative pathogens, respectively. ² - ; No inhibition zone ³ ND; Not determined.

4. DISCUSSION

A decontamination technique presented in this study provides a simple and cost-effective method as an alternative approach for eliminating microbial contamination and protecting the bioactive constituents of turmeric powder. The presence of important pathogens could potentially create a public health risk especially as the spice or herb is added at the end of cooking or to ready-to-eat foods that undergo no further processing (Chomnawang et al., 2003; Sagoo et al., 2009).

This study has shown that the turmeric powder obtained from clean non-decontaminated rhizome was of insufficient quality due to the presence of *Salmonella* spp. and high level of total aerobic microbial count, total combined yeasts and molds, and bile-tolerant Gram negative bacteria. Previous study in Thailand revealed that as much as 89% of non-registered herbal products did not conform to the THP standard (Chomnawang et al., 2003). This is extensively higher than the data reported from UK (Sagoo et al., 2009), Brazil (Moreira et al., 2009), Ireland (Witkowska et al., 2011) and Nigeria (Abba et al., 2009) where 4, 5, 20 and 46% of samples were of unacceptable quality, respectively. Nowadays, gamma radiation is the only decontamination technique recorded as a sufficient method for complete microbial eradication without affecting the bioactive properties of herbs. However, well-qualified and experienced staff as well as specially designed installation and equipment are required to perform this procedure (Mohammad et al., 2009). Because of these disadvantages, there is an urgent need to find other uncomplicated and cost-effective methods as an alternative strategy for disinfecting herbs and spices.

Our data indicates that four different inexpensive decontamination techniques including, soaking with 70% ethanol for 15 min, boiling in water for 15 min, boiling in 5% acetic acid for 15 min, and steaming at 121°C and 15 psi for 15 min could completely eliminate the microbial contamination in turmeric powder. There is however some previous information pointing out that high temperature decontamination may result in reduced bioactivity of herbal products (Suresh et al., 2007; Kumar et al., 2010). Therefore, the bioactive constituents of turmeric powder samples consisting of ethanol-and water-soluble extract, curcuminoids and volatile oils contents were further discussed.

According to the THP recommendation, the examined active principles of each turmeric sample were of suitable quality. It is interesting to note that, the amount of ethanol-and water-soluble extract, curcuminoids and volatile oils of these techniques were approximately two to three times higher than the amount recommended by the THP. Among the four evaluated processes, autoclaving at 121°C and 15 psi for 15 min was recorded as a promising decontamination procedure according to the highest contents of curcuminoids, volatile oils and ethanol-soluble extractive. Although, previous studies revealed that heat processing of turmeric results in a significant loss of their active principles (Srinivasan et al., 1992; Suresh et al., 2007). There have been some recent evidences supporting that turmeric is resistant to thermal denaturation (Temitope et al., 2010; Prathapan et al., 2009). The heat treatment of turmeric at 100°C for 1 h gives an increase in the total phenolic content, total flavonoids, and antioxidant activities (Temitope et al., 2010). Whereas, there was no significant change in the concentration of curcuminoids after the turmeric rhizome was subjected to heat treatment at 60-100°C for 10-60 min (Prathapan et al., 2009). Dried and cured turmeric yields 1.5 to 5% volatile oils which are dominated by sesquiterpenes and are responsible for its aromatic taste and smell. Boil points of ar-turmerone and turmerone, account for at least 40% of essential oils of turmeric are 125-126°C and 159-160°C, respectively. Previous investigation has been suggested that the compounds may be used to

control the product quality of turmeric oil (Li et al., 2011; Chempakam and Parthasarathy, 2008). Even though, the effect of decontamination processes of turmeric powder on active ingredients was similar, the antibacterial activity of each sample against skin and wound pathogens was comparable to previous investigations (Kim et al., 2005; Lawhavinit et al., 2010).

5. CONCLUSION

In conclusion, these findings suggest that the decontamination technique of fresh rhizome by high pressure streaming and further drying the sliced rhizome at 50°C for 48 h could be proposed as an effective, reliable and inexpensive procedure to decontaminate commercial turmeric powder without significant effecting in its active principles or biological activities. In order to improve the safety of herb and spices powder, the technique could now be investigated for application to different products.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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