Bacterial Contamination Assay of Thai Herbal Products

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Background: *Herbal products have been globally used for health promotion. These products were frequently contaminated with various microorganisms due to raw materials and unhygienic production process. Microbiological quality of several forms is necessary to determine by microbial contamination assay in order to assure the safety of Thai herbal products.*
Objective: *To investigate the microbiological quality of Thai herbal products commercially distributed from different regions of Thailand and marketed in Bangkok and Pathumthani province by bacterial contamination assay.*
Material and Method: *Thai herbal products were randomly collected in Bangkok and Pathum Thani province. Total of 94 samples were in different dosage forms including liquids, pills, capsules and powders. The samples were divided into two groups as Standard Certified and Non-Certified herbal products. The products were tested as plate count method using spread plate technique. The obtained results were interpreted based on Thai Herbal Pharmacopoeia.*
Results: *The total plate counts of Standard Certified samples were ranged between less than 30 to 5.8 x 10^7 cfu/g or ml and the Non-Certified samples were ranged between less than 30 and 6.0x10^6 cfu/g or ml. In addition, the results showed that 7 out of 49 (14.28%) of Standard Certified sample group and 5 out of 45 (11.11%) of the Non-Certified sample group were out of the range of acceptable standard. The finding indicated that the microbiological quality of both Standard Certified and Non-Certified Thai herbal products showed no significant differences.*
Conclusion: *Majority of the herbal products tested for bacterial contamination were in acceptable standard. However, some products were out of acceptable range. Hence the rigorous assessment of quality control and good manufactury practice of Thai herbal production is necessary for safe consumption.*

Keywords: *Bacterial contamination assay, Thai herbal products, Total plate counts, Standard Certified Thai herbal products, Non-Certified Thai herbal products*

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Thai herbal products have been produced from various parts of medicinal plants such as leaf, stem, root, flower and seed**.** Medicinal plants have been currently developed for many personal care products as they are natural. The elements containing herbs provide various biological activities for health promotion.

Various dosage forms of herbal products including as powder, tablets, capsules, gel and solutions are widely available in markets. Since herbal plants are natural ingredients contaminated from dust, sand, rock, grain, and feather, they are therefore easily contaminated by microorganisms resulting in acute diarrhea. Many herbal products remain unacceptable microbiological quality. The causative agents in herbal products including *Staphylococcus aureus*, *Clostridium* spp, *Salmonella* spp, *Shigella* spp and *Escherichia coli* have been previously reported[1,2]. The quality of products and safety of consumers was affected by these microorganisms. The microbial limitation for Thai herbal products such as powder, tablets and capsules must not exceed 5.0 x 10^5 cfu/g or ml of herbal product regarding to Thai Herbal Pharmacopoeia[3]. Therefore, Thai herbal products marketed in Bangkok and Phathum Thani province were evaluated for the microbiological quality by bacterial contamination assay.

In the present study, we examined 94 Thai herbal product samples including Standard Certified

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and Non-Certified products. The obtained results provided useful information for surveillance and prevention of bacterial contamination of Thai herbal products distributed commercially in Thailand. As a consequence, the manufacturers of Thai herbal products in Thailand will concern on rigorous quality control for safe consumption. Moreover, the rigid approval of Thai herbal products will be considered before commercially launch.

Material and Method

Total of 94 Thai herbal product samples from herbal shop, marketed in Bangkok, Phathumthani province and OTOP were randomly selected. Products included 49 Standard Certified and 45 Non-Certified products. Total plate count was performed with some modifications[4]. Briefly, herbal product sample of 10 g or 10 ml was mixed homogenously with 90 ml of sterile 0.9% normal saline solution. The sample was diluted in ten-fold dilution for six dilutions (10^{-1}-10^{-6}). The samples of each dilution (0.1 ml) were then spread on Plate Count Agar in duplicate and incubated at 37°C for 24-48 h. After incubation the growth of microbes were counted and calculated for total colony count (cfu/g or ml).

Results

Bacterial contamination assay of Thai herbal products from Bangkok and Pathum Thani province showed that the total aerobic microbial counts of all samples were between 30 cfu/g or ml to 5.8 x 10^7cfu/g or ml. In addition, 7 out of 49 (14.28%) of Standard Certified samples group were out of the range of acceptable standard (Fig. 1). The powder and tablet forms of Thai herbal products were out of acceptable standard (>5 x 10^5 cfu/g or ml). The average total aerobic microbial counts of the powder and tablet forms were 1.14 x 10^7 cfu/g and 8.15 x 10^6 cfu/g, respectively. Five out of 45 (11.11%) of the Non Certified sample group were out of the range of acceptable standard (Fig. 2). These samples were all in powder form. The total aerobic microbial count was 2.01 x 10^6 cfu/g. The majority of Thai herbal products examined in the present study was contaminated but still within the standard limit of Thai Herbal Pharmacopoeia. Moreover, total coliform bacterial assay of Thai herbal products showed that the preliminary test for faecal coliform of 18 samples was not detected (data not shown).

Discussion

Total of 94 Thai herbal products showed that 14.28% of Standard Certified samples and 11.11% of Non-Certified were out of the range of acceptable standard. The Standard Certified Thai herbal products in this study exhibited more contamination than certified Thai traditional medicine distributed in Khon Kaen province (10.3%)[5]. The herbal products from all regions of Thailand reported by Chomnawang and colleagues showed 87.72% of Non-Certified products were out of the range of acceptable standard[6]. It is denoted that the herbal products from several provinces distributed in Bangkok and Phathumthani province had better quality than previous report[6]. This obtained results indicated that the production of herbal products of manufactureres was under an efficient quality control and good manufacturing practice.

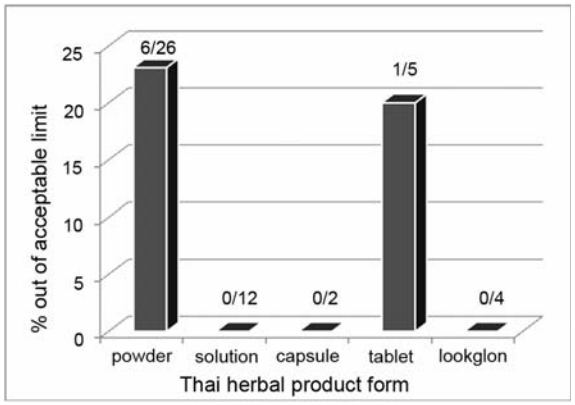

Fig. 1 Total plate count of 49 Standard Certified samples. Each bar represented the percentage of samples containing total microbial count more than maximum limitation of Thai Herbal Pharmacopoeia

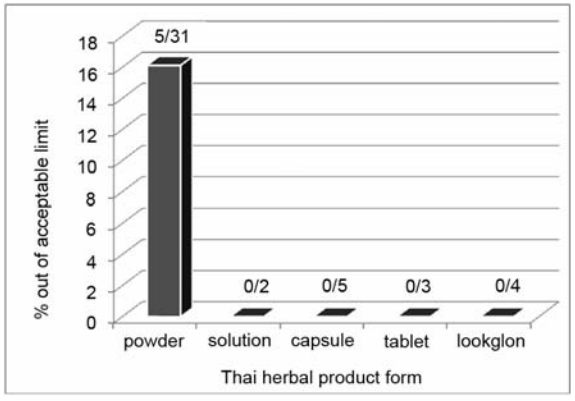

Fig. 2 Total plate count of 45 Non-Certified samples. Each bar represented the percentage of samples containing total microbial count more than the maximum limitation of Thai Herbal Pharmacopoeia

Khanyile and colleagues showed that the commercial herbal products of dried powder leaves were contaminated with harmful bacteria whereas the different commercial product such as adaptogenic tonic was free from bacterial contamination. In addition, the average of total bacterial count of 9.5 x 10^4 cfu/g found in powder leafs extracts[7]. In contrast, the powder form of the Thai herbal products in the present study showed total bacterial count of 5.8 x 10^7cfu/g. It is suggested that the contamination of microorganism found in powder form of Thai herbal products is likely resulted from poor storage of the products. In addition the powder and tablet forms of herbal products seemed to have more contamination than other forms. The authors suggest that the contamination found in powder and tablet forms is possibly due to the effects of moisture and temperature during storage. Moreover, the products containing spices and herbs with high level of microbial contamination were caused by contaminated spices and herbs in the process of production, processing and usage of the products.

Surveillance and control of herbal products manufacture was necessary for prevention of food-borne diseases[8]. Esimone and colleagues examined herbal medicinal products marketed in mid Western Nigeria showed that all samples were contaminated with various bacterial strains such as *Bacillus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp and *Streptococcus* spp[9]. In addition, *Salmonella* spp was detected in some herbal solid dosage forms[10].

Interestingly, the Thai herbal products in Lookglon form showed average total aerobic microbial counts of 8.6 x 10^3cfu/g. It was in the acceptable standard range in spite of poor packaging of the products observed. This obtained results indicated that the product in Lookglon form was likely to be processed with good manufacture practice including the packaging process. In addition, Lookglon form usually contained honey which may result in inhibition of bacterial growth. The combination of herbal components in herbal Thai products is another possible factor which related to the antibacterial activity against bacteria contaminated in the products. However, Lookglon form of Thai herbal products is recommended to handle with careful process of production to avoid bacterial contamination.

The total bacterial count of most Thai herbal products was in the acceptable microbiological criteria as mentioned earlier. However, this conventional method may not be able to discover the pathogenic bacteria effectively. The detection of pathogenic bacteria including *E. coli*, *Salmonella* spp, *Shigella* spp and *S. aureus* with small numbers of the bacteria will be further investigated for more insightful detailed information of the Thai herbal products quality. Molecular method such as polymerase chain reaction for the pathogenic bacterial contamination will be approached in future.

Conclusion

The present study was signified that mostly manufacturers produced herbal products in acceptable standard and good quality control. However, some samples were found to be contaminated and out of the acceptable range. The contaminated bacteria could be pathogenic microorganism leading to human diseases such as acute diarrhea. Therefore, the Thai herbal products must be under the rigorous assessment of quality control and good manufacturing practice. The obtained results of this study emphasized the necessity of improving plant material quality and establishing better hygienic conditions of Thai herbal production.

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Potential conflicts of interest

None.

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ทดสอบการปนเปื้อนเชื้อแบคทีเรียในผลิตภัณฑ์สมุนไพรไทย

พิมลวรรณ โภคาพันธ์, สุมาลี คอนโด

ภูมิหลัง: *ผลิตภัณฑ์สมุนไพรเพื่อสุขภาพได้ถูกนำมาใช้กันอย่างแพร่หลายทั่วโลก กลุ่มผลิตภัณฑ์เหล่านี้พบว่ายังมีการปนเปื้อนจุลชีพต่างๆ มากมายซึ่งสาเหตุการปนเปื้อนนั้นมาจากวัตถุดิบและขบวนการผลิตที่ไม่ถูกสุขลักษณะ จึงจำเป็นที่จะต้องมีการตรวจสอบคุณภาพและความปลอดภัยของการปนเปื้อนจุลชีพในผลิตภัณฑ์สมุนไพรไทย*

วัตถุประสงค์: *เพื่อตรวจสอบคุณภาพทางชีววิทยาของผลิตภัณฑ์สมุนไพรที่ผลิตจากภาคต่างๆในประเทศไทย และนำมาขายในพื้นที่กรุงเทพมหานครและจังหวัดปทุมธานี*

วัสดุและวิธีการ: *สุ่มเก็บตัวอย่างผลิตภัณฑ์สมุนไพรไทยจำนวน 94 ตัวอย่าง ในเขตกรุงเทพมหานครและปทุมธานี ซึ่งรูปแบบของผลิตภัณฑ์แตกต่างกันไปเช่น น้ำ เม็ด แคปซูลและผง โดยแบ่งตัวอย่างออกเป็น 2 กลุ่มคือ กลุ่มที่มีมาตรฐานรับรองและกลุ่มที่ไม่มีมาตรฐานรับรอง จากนั้นนำตัวอย่างไปทดสอบการปนเปื้อนของเชื้อแบคทีเรียด้วยการใช้เทคนิค spread plate แปลผลตามเกณฑ์มาตรฐานทางด้านจุลชีววิทยาที่เภสัชตำรับไทยกำหนด*

ผลการศึกษา: *ปริมาณการปนเปื้อนแบคทีเรียโดยรวมของกลุ่มผลิตภัณฑ์สมุนไพรที่มีมาตรฐานรับรองมีค่าระหว่าง < 30-5.8 x 10^7 cfu/g หรือ ml ส่วนกลุ่มผลิตภัณฑ์สมุนไพรที่ไม่มีมาตรฐานรับรองมีค่าระหว่าง < 30–6.0x10^6 cfu/g or ml นอกจากนี้ พบว่าจำนวนผลิตภัณฑ์สมุนไพรที่มีมาตรฐานรับรองมีการปนเปื้อนอยู่ในระดับที่ไม่ผ่านเกณฑ์มาตรฐานคิดเป็นร้อยละ 14.28 ส่วนจำนวนผลิตภัณฑ์สมุนไพรที่ไม่มีมาตรฐานรับรองมีการปนเปื้อนอยู่ในระดับที่ไม่ผ่านเกณฑ์มาตรฐาน คิดเป็นร้อยละ 11.11 การศึกษานี้แสดงให้เห็นว่าคุณภาพทางจุลชีววิทยาของผลิตภัณฑ์สมุนไพรไทยที่มีมาตรฐานรับรอง และไม่มีมาตรฐานรับรองไม่มีความแตกต่างอย่างมีนัยสำคัญ*

สรุป: *ผลิตภัณฑ์สมุนไพรส่วนใหญ่ที่ทดสอบการปนเปื้อนอยู่ในระดับมาตรฐานการยอมรับ อย่างไรก็ตามผลิตภัณฑ์สมุนไพรบางชนิด มีระดับการปนเปื้อนที่ไม่อยู่ในระดับมาตรฐานการยอมรับ ดังนั้นการประเมินการควบคุมคุณภาพการผลิต ผลิตภัณฑ์สมุนไพรไทยที่เข้มงวด จึงมีความจำเป็นเพื่อความปลอดภัยในการบริโภคผลิตภัณฑ์สมุนไพรดังกล่าว*