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Microbial contamination of turmeric capsules produced at a secondary government hospital in the northeastern Thailand

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Objectives: The aim of this study was to assess the microbial quality of turmeric capsules produced at a secondary government hospital in the northeastern Thailand following the Thai Herbal Pharmacopoeia.
Methods: The six lots of turmeric capsules that produced not more than three months were included in this study. The total aerobic microbial and total combined yeasts and molds were counted. Contamination of coliform bacteria, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* spp. and *Clostridium* spp. were also investigated using selective media.
Results: All six lots of turmeric capsules had exceeded amount of total aerobic microbial count and total combined yeasts and molds count. However, coliform bacteria was less than 3 MPN/g. *E. coli, S. aureus, P. aeruginosa, Salmonella* spp., and *Clostridium* spp. were absence.
Conclusion: All six lots of turmeric capsules were failed the microbial quality of the Thai Herbal Pharmacopoeia. Thus, microbial contamination of turmeric capsules produced at this hospital should be improved.

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Introduction

Curcuma longa L. or turmeric is the edible and medicinal plant belonging to the ginger family, Zingiberaceae. When turmeric rhizome is dried and pulverized, a yellow powder with a bitter, slightly acrid, and yet sweet taste obtained.[1] In Thailand, it is used as a spice in Thai food and herb in Thai traditional medicines for treatment of flatulence and peptic ulcer. The major active constituent of turmeric rhizome is curcuminoids and volatiles oil. The curcuminoids include three compounds; curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Furthermore, turmerone is commonly found as a major volatile compound.[1] A turmeric capsule is officially include in the 2013 National Essential Drug List for treatment of flatulence.[2] However, a turmeric rhizome has a numerous pharmacological effects on various diseases such as treatment of arthritis[3], irritable bowel syndrome[4], inflammatory bowel disease[5, 6], peptic ulcer[7], cancer[8], pancreatitis[9], etc. Some publications reported that microbial contamination of herbal products in Thailand is continuously found. Chomnawang et al. informed that 50 of 57 non-registered herbal products samples selected from all over regions of Thailand did not conformed to the standard of the Thai Herbal Pharmacopoeia (THP) in the topic of microbial limit test.[10] Pocachon and Kondo reported that some Thai herbal products have bacterial contamination higher than 10% of products marketed in different regions of Thailand.[11] In addition, Boonruad et al. reported chemical quality of turmeric raw materials and herbal capsules produced in regional hospitals. Result showed that 42.5% of 40 samples of turmeric raw materials and 22.9% of 35 samples of turmeric capsules meet the standard specification of the THP. This result indicated the turmeric raw materials and capsules required the improvement of their chemical quality.[12]
The Thai Ministry of Public Health promoted the production of herbal medicinal products in hospital in order to use instead of some modern medicinal products, thus some government hospitals produced herbal products by themselves. As the herbal products officially included in the National Essential Drug List, turmeric capsule is the most popular herbal product that produced and dispensed to the patients. However, quality control of this herbal product is infrequently assessed. This study was to assess the microbiological quality of six lots of turmeric capsules produced at a secondary government hospitals in the northeastern Thailand. Evaluation topics are investigated following the standard specification of the THP.

Methods

***Turmeric capsule samples*:** Six lots of turmeric capsules were obtained from a secondary government hospital in the northeastern Thailand. Turmeric capsules that produced less than three months were included in this study. The

turmeric powder was removed from turmeric capsules and subsequently used for microbial contamination test.

Total aerobic microbial count: Ten grams of turmeric powder was diluted to obtain 1:10, mixed, and serial 10-fold diluted. One mL sample and 20 mL plate count agar were added into Petri dish (n=3), mixed by swirling and allow to solidify, and incubated at 35±1 ºC for 48 h. Petri dish that had 25-250 colonies was selected and counted. Average value of colonies number was multiplied by dilution factor. A colony-forming unit (CFU) per gram of sample was reported.

Total combined yeasts and molds count: Ten grams of turmeric powder was diluted at ratio of 1:10, 1:100, and 1:1000. The 0.1 mL sample was spread on Petri dish containing solidified potato dextrose agar (n=3). Then, incubated at 20-25 ºC for 2-7 days. Colonies number was counted and CFU per gram of sample was reported.

Test for coliform bacteria: Ten grams of turmeric powder was diluted at ratio of 1:10, 1:100, and 1:1000. One mL sample was added to test tube containing lauryl tryptose broth and Durham tube. Coliform bacteria was tested in triplicate for each dilution sample. The obtained samples were incubated at 35±1 ºC. After 24±2 h, sample that showed gas production in Durham tube was collected. Conversely, sample with gas-free Durham tubes were continue incubated for 48±2 h. Gas production sample was subcultured into brilliant green lactose bile broth and incubated at 35±1 ºC for 48±2 h. The Most Probable Number (MPN) of coliform bacteria per gram of sample was reported.

Test for Escherichia coli: In case of coliform bacteria found in brilliant green lactose bile broth, the obtained broth was subcultured into eosin-methylene blue agar and incubated at 30-35 ºC for 18-24 h. The obtained colonies were compared to colonies of test *E. coli*. Furthermore, gram stain was performed, if necessary.

Test for Staphylococcus aureus: Ten grams of turmeric powder was mixed with 100 mL tryptic soy broth and incubated at 30-35 ºC for 18-24 h. The obtained sample was subcultured into mannitol salt agar and incubated at 30-35 ºC for 18-72 h. The obtained colonies were compared to colonies of test *S. aureus*. Furthermore, gram stain was performed, if necessary.

Test for Pseudomonas aeruginosa: Ten grams of turmeric powder was mixed with 100 mL tryptic soy broth and incubated at 30-35 ºC for 18-24 h. The obtained sample was subcultured into cetrimide agar and incubated at 30-35 ºC for 18-72 h. The obtained colonies were compared to colonies of test *P. aeruginosa*. Furthermore, gram stain was performed, if necessary.

Test for Salmonella spp.: Ten grams of turmeric powder was mixed with 100 mL tryptic soy broth and incubated at 30-35 ºC for 18-24 h. The 0.1 mL obtained sample was added to 10 mL Rappaport Vassiliadis broth. And, the 1 mL obtained sample was added to 10 mL tetrathionate brilliant-green bile enrichment broth. Both samples were incubated at 30±1 ºC for 18-24 h. The obtained sample was subcultured into xylose-lysine-deoxycholate agar, then, incubated at 30-35 ºC for 18-48 h. The obtained colonies were compared to colonies of test *Salmonella* spp. Furthermore, gram stain was performed, if necessary.

Test for Clostridium spp.: Ten grams of turmeric powder was mixed with 100 mL tryptic soy broth and incubated at 30-35 ºC for 18-24 h. The 1 mL obtained sample was added to 2 tubes of 10 mL reinforced Clostridial medium. The first tube was heated at 80 ºC for 10 min and allow to cool immediately. But, the second tube was non-heated. Mineral oil was added into both two tubes and incubated at 30-35 ºC for 48 h. Each obtained sample was subcultured into Columbia blood agar containing gentamicin sulfate and incubated at 30±1 ºC for 48-72 h under anaerobic condition. If no bacteria growth, the sample was reported as absence of *Clostridium* spp.

Results

All six lots of turmeric capsules had high amount of total aerobic microbial. Microbial count was higher than 150 colonies for all dilutions, thus “too numorous to count” was reported for all six lots. All six lots had total combined yeasts and molds count ranged from 4.3-41.0 CFU/g, which, lot 2 and lot 6 showed the higheast and lowest value, respectively. Result of total combined yeasts and molds count is shown in figure 1.

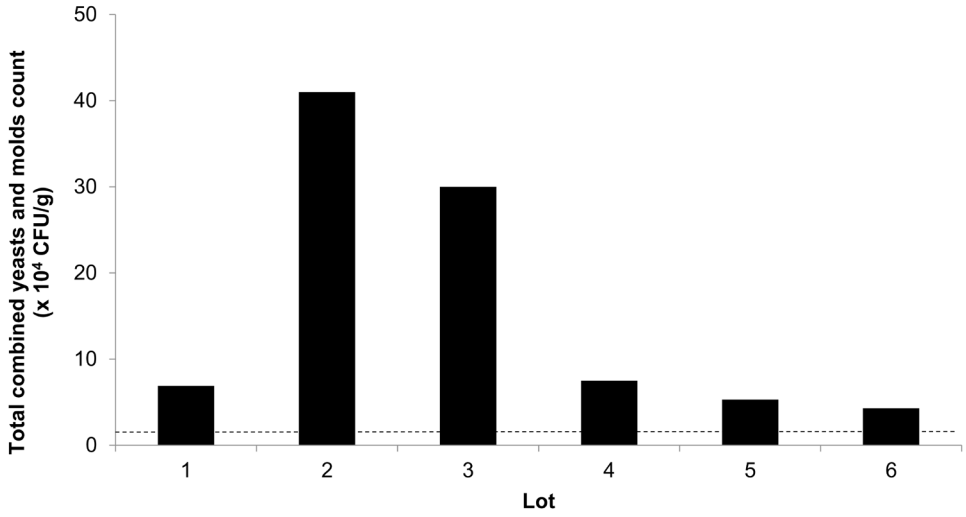

Figure 1. Total combined yeasts and molds count. Dashed line indicated standard criteria officially included in the THP.

Other result of microbial limit test is shown in Table 1. All six lots of turmeric capsules had coliform bacteria lower than 3 MPN/g. Pathogenic bacterias such as *E. coli*, *S. aureus*, *P. aeruginosa*, and *Clostridium* spp. were absence in 1 g

sample, Moreover, *Salmonella* spp. was absence in 10 g sample.

Table 1. Result of microbial limit test of six lots of turmeric capsules

Lot	Coliform	*E. coli*	*S. aureus*	*P. aeruginosa*	*Salmonella* spp.	*Clostridium* spp.
1	< 3 MPN/g	Absence	Absence	Absence	Absence	Absence
2	< 3 MPN/g	Absence	Absence	Absence	Absence	Absence
3	< 3 MPN/g	Absence	Absence	Absence	Absence	Absence
4	< 3 MPN/g	Absence	Absence	Absence	Absence	Absence
5	< 3 MPN/g	Absence	Absence	Absence	Absence	Absence
6	< 3 MPN/g	Absence	Absence	Absence	Absence	Absence

Discussion

The THP (2009) specified that total aerobic microbial count, total combined yeasts and molds count, and bile-tolerant gram negative bacteria of preparations for internal use containing ground crude drugs should be not more than 2×10^5 CFU/g, 2×10^4 CFU/g, and 10^3 /g, respectively. Furthermore, *E. coli* per 1 g sample, *Clostridium* spp. per 1 g sample, and *Salmonella* spp. per 10 g sample should be absence.[13] However, bile-tolerant gram negative bacteria was no test in this work.

This work showed that all six lots of turmeric capsules had total aerobic microbial count and total combined yeasts and molds count higher than 2×10^5 and 2×10^4 CFU/g, respectively. This result indicated that all six lots of turmeric capsules failed the standard criteria of the THP. However, tests for pathogenic bacteria such as *E. coli*, *Clostridium* spp., and *Salmonella* spp. were met the standard criteria of the THP. This results were similar to other publication that turmeric capsules usually contained exceed amount of total aerobic microbial and total combined yeasts and molds.[14]

Many factors such as agriculture, harvest, production process, transportation, and storage are affected the quality of herbal products both physical, chemical, and microbiological manner. Turmeric capsules produced at these secondary hospital was used turmeric that planted by people in their provinces. After turmeric rhizomes were harvested, it was cleaned, dried, and ground. Finally, it was filled into capsules, packing, and storage. Thus, all process affected the microbial quality of turmeric capsules. Because of part used of turmeric was rhizome that usually contaminated with natural microbial in soil, thus well-organized cleaning process, removed of rhizome shell, or treated with boiling water before ground should be used to reduced microbial contamination. Gamma irradiation was one of the highly effective sterilization procedure for herbal products.[15] Thus, gamma irradiation should be applied for terminal sterilization of turmeric capsules to improved microbial quality of their herbal products.

Conclusion

The six lots of turmeric capsules produced at a secondary government hospital in the northeastern Thailand were evaluated for their microbial quality following standard criteria of the THP. Unfortunately, all six lots of turmeric capsules had exceeded amount of total aerobic microbial count and total combined yeasts and molds count. But, pathogenic bacteria was absence. Thus, turmeric capsules produced in these hospital should be improved the microbial quality.

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