MICROBIAL, STEROIDS AND HEAVY METALS CONTAMINATION AND ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES IN THAI HERBAL PRODUCTS

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Abstract. Screening for the presence of bacteria, steroids, and heavy metals is essential for the protection of consumers of herbal medications. Samples of Thai herbal medicine (n = 59) were collected from one metropolitan region and four provinces to test for the presence of bacteria, steroids and heavy metal contamination. Total aerobic microbial and total yeast/mold counts ranged from 0 - 9×10^6 and 0 - 20 colony-forming units (CFU)/g respectively. The range of 0 – 1,100 CFU/g represented the most likely coliform bacterial concentrations. *Clostridium* spp, *Escherichia coli* and *Pseudomonas aeruginosa* were detected in 19, 14 and 3 percent of the samples, respectively, whereas *Salmonella* spp and *Staphylococcus aureus* were not present. Overall, 47% of the herbal medicine samples fulfilled the standards for quality set forth by the WHO guidelines and Thai Pharmacopoeia. The percentage of samples that met the quality standards was 100, 64, 50, 50, 40, 33, and 0 percent for tea, tablets, capsules, liquids, pills, and powder forms, respectively. While all *P. aeruginosa* isolates were sensitive to the seven test antibiotics, 25% of *E. coli* isolates demonstrated multidrug resistance, namely, resistance to amoxicillin/clavulanate, ampicillin, ciprofloxacin, and trimethoprim/sulfamethoxazole. PCR-based assays revealed that all *E. coli* and *P. aeruginosa* isolates carried bla_{TEM} but not bla_{SHV} gene, while 25% of *E. coli* also contained $bla_{\text{CTX-M}}$ gene. An immunochromatographic assay revealed that 2% of the herbal medicine samples were positive for dexamethasone (confirmed by a reference laboratory of the Ministry of Public Health Thailand) and paracetamol and diclofenac (non-steroid anti-inflammatory drugs) were also detected. The mean ± standard deviation (SD) (range) of cadmium and lead evaluated in 32/59 randomly selected samples using in-house methods based on AOAC

International (2016) 999.10 was 0.0385 ± 0.0247 mg/kg (0.003-0.264 mg/kg) and 0.140 ± 0.009 mg/kg (0.003-0.617 mg/kg) respectively, within the limit of cadmium and lead standard criterion (≤0.3 and ≤10 mg/kg respectively). The study's findings should contribute to raising the standard of Thai herbal medicine preparations.

Keywords: heavy metal, Thai herbal medicine, microbe, steroid

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INTRODUCTION

Based on a survey by the World Health Organization (WHO), approximately 60-80% of the world population use herbal medicine, mostly derived from plants (Chen *et al*, 2021). Improvements in analysis and quality control, along with progress in clinical research indicate the value of herbal medicine in the prevention and treatment of diseases (Mohamed *et al*, 2012). Traditional herbal medicine consists of natural plant-based substances that have undergone little or no industrial processing and have been accepted for use in local and regional healing practices to treat diseases. More than 87% of raw materials in Chinese medicine are derived from plants, such as the underground (roots, rhizomes) and the above-ground (stems, flowers, fruits, seeds) parts, and even the entire plant itself (Chen *et al*, 2021). Heavy metals, such as lead and cadmium, are primarily present respectively in the leaves and in the flowers and fruits (Chen *et al*, 2021).

Herbal medicine is used either as a stand-alone treatment or as complementary therapy alongside conventional medicine due to tradition, previous positive experience, and/or low cost. Screening of safety in the use of herbal medicine is crucial and involves assessing such factors as the presence of microbes (pathogenic bacteria, such as *Clostridium* spp, *Pseudomonas aeruginosa*, *Salmonella* spp, and *Staphylococcus aureus*, and fungi), steroids and heavy metals

(WHO, 2011; Cicero *et al*, 2022). Regulations on herbal medicine are usually promulgated as rules and/or laws designed to control or govern the quality of products from manufacturers and producers (WHO, 2019).

WHO (2011), the Thai Pharmacopoeia (DMSC-MOPH, 2017) and a recent report by Kalumbi *et al* (2020) recommend a limit of 10^3 colony forming units (CFU) (enterobacteria)/g medicinal plant material intended for internal use. In addition, it is necessary that herbal medicine not contain any pathogenic bacteria and steroids. The presence of cadmium and lead should be <0.3 and <10 mg/kg respectively (DMSC-MOPH, 2017).

The Ministry of Public Health Thailand's regulation in 2021 on contaminations of raw materials and final herbal medicinal products intended for use, limit the total aerobic microbial (TAMC) and yeast and mold counts (TYMC) of tea (for brewing in boiling water) to $<5.0 \times 10^7$ and $<5.0 \times 10^5$ CFU/g or CFU/ ml respectively while for other products, the TAMC and YMC limit is $<5.0 \times 10^4$ and $<5.0 \times 10^2$ CFU/g or CFU/ml, respectively (MOPH, 2021). For raw materials and herbal medicine capsules, the Medicinal Plant Research Institute, Department of Medical Sciences, Ministry of Public Health Thailand limits TAMC and TYMC to $<5.0 \times 10^7$ and $<5.0 \times 10^3$ CFU/g or CFU/ml respectively (DMSC-MOPH, 2005).

A survey of 25 samples of five different Chinese herbs available in Thailand showed that only 64% passed quality standards (total bacterial counts, total combined yeast and mold counts and absence of *Salmonella* spp and *S. aureus*) (Rangsipanuratn *et al*, 2017). In Kenya, Onyambu *et al* (2013) reported that unregulated herbal medicine has poor microbial quality control, with the presence of pathological bacterial contaminations, *eg Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Salmonella* spp, and *S. aureus*.

As regards to steroids contamination, in Singapore Fung and Linn (2017) reported the presence of various steroids (betamethasone, dexamethasone and prednisolone) in illegal herbal medicine. In Thailand, 23% of traditional medicine samples ($n = 100$) in Bangkok were adulterated with steroids (Chaiyasothi *et al*, 2014) and, more recently, in Nakhon Pathom Province, 32% ($n = 90$) contained steroids (dexamethasone and/or prednisolone) (Watsanaphitranon *et al*, 2021).

As for heavy metal contamination in herbal medicine, in Thailand all 25 samples of five Chinese herbs were contaminated with arsenic, cadmium and lead, but within permissible levels (Rangsipanuratn *et al*, 2017). In a systematic review of the literature Chen *et al* (2021) reported the presence of arsenic, cadmium, chromium, lead, and mercury in various Chinese traditional medicinal materials, with the highest levels of metal residues in samples from Qinghai-Tibet Plateau and south and southwest China.

Thus, the aim of this study was to determine the presence of microbes (pathogenic bacteria and fungi), steroids and heavy metals in herbal medicine in Thailand. Information regarding antimicrobial resistance among the bacterial isolates including carriage of drug resistance genes will provide baseline data on the current status of contamination in Thai herbal products, which should assist in monitoring the safety of herbal medicine in the country.

MATERIALS AND METHODS

Thai herbal medicine samples

Fifty-nine samples were collected from Bangkok and four provinces in Thailand (Phra Nakhon Si Ayutthaya, Nakhon Pathom, Nonthaburi, and Prachuap Khiri Khan) between 01 October 2021 and 30 April 2022. The study protocol was approved by the Human Research Ethics Committee, Faculty of Pharmacy, Siam University (approval no. SIAMPY-IRB 2022/003).

Microbial determination (bacteria and yeasts/molds)

The total aerobic microbial (TAMC), yeast and mold (TYMC) counts, and the numbers of bacterial and coliform bacteria isolates, including identification of specific bacteria (*Clostridium* spp, *E. coli*, *P. aeruginosa*, *S. aureus*, and *Salmonella* spp) in the samples were determined by previously described methods (WHO, 2011; Strockbine *et al*, 2015; de Sousa Lima *et al*, 2020; Cicero *et al*, 2022).

Antimicrobial susceptibility assay

Each bacterial isolate was kept at -70°C in 5% trypticase soy broth with 20% (v/v) glycerol (Oxoid, Hampshire, UK) until used. The assay employed a standard disk diffusion method, according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2021). Each disk (Oxoid, Hampshire, UK and Becton Dickinson, Franklin Lakes, NJ) contained amikacin (AK, 30 μg), ampicillin (AP, 10 μg), AUG amoxicillin/clavulanic acid (AUG, 20/10 μg), cefoxitin (FOX, 30 μg), cefuroxime (CXM, 30 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), cefepime (FEP, 30 μg), ciprofloxacin (CIP, 5 μg), gentamycin (G, 10 μg), imipenem (IMP, 10 μg), netilmicin (NET, 30 μg), piperacillin/tazobactam (TZP, 100/10 μg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains. A multidrug-resistant bacterial isolate is defined as resistance to ≥3 classes of antibiotics (Seng *et al*, 2018).

Molecular assay of drug resistance genes

A PCR-based assay was used to detect $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} as previously described (Oliver *et al*, 2002; Pitout *et al*, 2007). Specific primers were CTX-M-forward (5′ SCSATGTGCAGYACCAGTAA 3′) and CTX-M-reverse (5′ CCGCRATATGRTTGGTGGTG 3′) (amplicon size 544 bp), TEM-forward (5′ ATGAGTATTCAACATTTCCG 3′) and TEM-reverse (5′ CTGACAGTTACCAATGCTTA 3′) (amplicon size 867 bp) and SHV-forward (5′ GGTTATGCGTTATATTCGCC 3′) and SHV-reverse (5′ TTAGCGTTGCCAGTGCTC 3′) (amplicon size 867 bp), where R = A or G; S = G or C and Y = C or T. Thermal cycling conditions, using Thermal Cyclers for PCR Machine (Bio-rad, Hercules, CA), were as follows: 95°C for five minutes, followed by 30 cycles of 95°C for 30 seconds, 55°C ($bla_{\text{CTX-M}}$, bla_{TEM}) or 56°C (bla_{SHV}) for 60 seconds and 72°C for 60 seconds, and a final step at 72°C for five minutes. Amplicons were analyzed by 2% agarose gel-electrophoresis at 77 V for 45 minutes. Gels were then stained with ethidium bromide and bands recorded using a gel documentation system machine (Syngene, London, UK).

Determination of steroids

Steroid test kits (immunochromatographic method) were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. The assays were carried out as previously described (Matapatara and Likitthanaset, 2010; Chaiyasothi *et al*, 2014). If the result was positive, the sample was sent for confirmation of steroid identity at the reference laboratory, Narcotics Control Division, Ministry of Public Health, Thailand.

Determination of heavy metals

The analysis for cadmium and lead in 32/59 randomly selected samples was carried out by the Medical and Food Lab Co Ltd (Bangkok, Thailand) according to AOAC Official Method 999.10 (AOAC International, 2016).

Data analysis

Category variables were expressed as percentage, mean ± standard deviation, or range. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp, Armonk, NY).

RESULTS

A total of 59 samples of Thai herbal medicine were collected from one metropolitan area and four provinces: Phra Nakhon Si Ayutthaya Province (*n* = 8, 14%), Bangkok (*n* = 39, 66%), Nakhon Pathom Province (*n* = 2, 3%), Nonthaburi Province (*n* = 3, 5%), and Prachuap Khiri Khan Province (*n* = 7, 12%). The samples were in seven forms: capsule (*n* = 18), liquid (*n* = 4), tablet (*n* = 14), tea (*n* = 4), pill (*n* = 5), powder (*n* = 8), and raw material (*n* = 6) (Table 1). The total aerobic microbial (TAMC) and total yeast/mold (TYMC) counts ranged from 0 - 9×10^6 and 0 - 20 colony forming unit (CFU)/g respectively. The presence of coliform bacteria ranged from 0 - >1,100 CFU/g. *Clostridium* spp, *E. coli* and *P. aeruginosa* was detected in 19, 14 and 3 percent of the samples respectively, but *S. aureus* and *Salmonella* spp were not detected (Table 1). Overall, nearly half (47%) of the herbal medicine samples passed the quality standards for TAMC and TYMC set by WHO (2011) and Thai Pharmacopoeia (DMSC-MOPH, 2017). When assessed according to types of samples, compliance for capsule, liquid, tablet, tea, pill, powder, and raw material was 50, 50, 57, 100, 40, 0, and 33 percent, respectively (Table 1).

Of the three pathogenic bacteria detected, 25% of *E. coli* isolates exhibited multidrug resistance phenotype, *ie* resistant to amoxicillin/clavulanate, ampicillin, ciprofloxacin, and trimethoprim/sulfamethoxazole, while the two *P. aeruginosa* isolates were susceptible to all seven test antibiotics (Table 2). A PCR-based assay showed that all *E. coli* and *P. aeruginosa* isolates carried bla_{TEM} gene, 25% of *E. coli* isolates (different from the two multi-drug resistant isolates) carried $bla_{\text{CTX-M}}$ gene [resistance to cefuroxime

Table 1

Microbes and coliform counts in herbal medicine samples

Herbal medicine sample[a]	TAMC (CFU/g)	TYMC (CFU/g)	MPN (/100 ml)	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella* spp.	*Clostridium* spp.	Quality standard
Tea (n = 4)									
Cathamus tinctorius[b]	0	0	<3.0	-	-	-	-	-	Pass
Senna leaf tea[b]	0	0	<3.0	-	-	-	-	-	Pass
Orthosiphon aristatus[b]	0	0	3.6	-	-	-	-	-	Pass
Green tea	18,000	0	9.2	-	-	-	-	-	Pass
Tablet (n = 14)									
Cough herb-5	0	0	28	-	-	-	-	-	Pass
Cough herb-6	6,000	0	23	-	-	-	-	-	Fail
Andrographis paniculata-4[b]	0	0	35	-	-	-	-	-	Pass
Ya tip osod[b]	0	0	<3.0	-	-	-	-	-	Pass
Antipyretic[b]	0	0	<3.0	-	-	-	-	-	Pass
Stomach herb-2[b]	0	0	<3.0	-	-	-	-	-	Pass
Cough herb-7[b]	0	0	<3.0	-	-	-	-	-	Pass
Ya sahasthara[b]	0	0	<3.0	-	-	-	-	-	Pass
Triphala[b]	45,000	0	<3.0	-	-	-	-	-	Pass
Ya keow	600,000	0	>1,100	+	-	-	-	-	Fail
Bitter herb	105,000	0	1,100	-	-	-	-	-	Fail

Table 1 (cont)

Herbal medicine sample[a]	TAMC (CFU/g)	TYMC (CFU/g)	MPN (/100 ml)	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella* spp.	*Clostridium* spp.	Quality standard
Tablet (n = 14)									
Cough herb-4	750,000	0	240	-	-	-	-	-	Fail
Cough herb-8	150,000	0	210	-	-	-	-	-	Fail
Cough herb-9	105,000	0	75	-	-	-	-	-	Fail
Capsule (n = 18)									
Andrographis paniculata-1[b]	0	0	<3.0	-	-	-	-	-	Pass
Jiaogulan[b]	50,000	0	460	-	-	-	-	-	Pass
Thai herb-2[b]	9,000	0	11	-	-	-	-	-	Pass
Andrographis paniculata-3[b]	30,000	0	23	-	-	-	-	-	Pass
Boesenbergia rotunda-2[b]	0	0	23	-	-	-	-	-	Pass
Derris scandens-2[b]	0	0	<3.0	-	-	-	-	-	Pass
Boesenbergia rotunda-3[b]	9	0	23	-	-	-	-	-	Pass
Andrographis paniculata-5[b]	0	0	<3.0	-	-	-	-	-	Pass
Andrographis paniculata-6[b]	0	0	<3.0	-	-	-	-	-	Pass
Curcuma comosa	600,000	0	<3.0	-	-	-	-	-	Fail

Table 1 (cont)

Herbal medicine sample[a]	TAMC (CFU/g)	TYMC (CFU/g)	MPN (/100 ml)	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella* spp.	*Clostridium* spp.	Quality standard
Capsule (*n* = 18)									
Fat lose pill	750,000	1	1,100	-	-	-	-	-	Fail
Andrographis paniculata-2	186,000	0	>1,100	-	-	-	-	+	Fail
Derris scandens-1	9,000,000	0	>1,100	-	-	-	-	+	Fail
Turmeric-2	6,000,000	0	43	-	-	-	-	+	Fail
Thunbergia laurifolia	600,000	1	3.6	+	-	-	-	+	Fail
Boesenbergia rotunda-1	300,000	0	23	+	-	-	-	+	Fail
Stomach herb-1[b]	60,000	0	290	-	-	-	-	+	Fail
Ya harak	90,000	20	240	-	-	-	-	-	Fail
Liquid (*n* = 4)									
Cough herb-2[b]	6,000	0	<3.0	-	-	-	-	-	Pass
Cough herb-3[b]	0	0	<3.0	-	-	-	-	-	Pass
Cough herb-1[b]	480,000	0	35	-	-	-	-	-	Fail
Kaffir lime[b]	1,200,000	0	3	-	-	-	-	-	Fail

Table 1 (cont)

Herbal medicine sample[a]	TAMC (CFU/g)	TYMC (CFU/g)	MPN (/100 ml)	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella* spp.	*Clostridium* spp.	Quality standard
Pill (*n* = 5)									
Ngueak pla mo-*Acanthus ebracteatus*	30,000	0	43	-	-	-	-	-	Pass
Diet pill	21,000	0	<3.0	-	-	-	-	-	Pass
Ya sinus herb	0	0	21	-	-	-	-	+	Fail
Always Young Woman herb	0	0	93	-	-	-	-	+	Fail
Ya khlay zen	9,000	0	3.6	+	-	-	-	-	Fail
Raw material (*n* = 6)									
Black pepper	360,000	2	<3.0	-	-	-	-	-	Pass
Long pepper	0	0	<3.0	-	-	-	-	-	Pass
Coriander seeds	600,000	20	>1,100	-	-	+	-	+	Fail
Bitter gourd	600,000	0	>1,100	-	-	-	-	-	Fail
Senna leaf	15,000	0	>1,100	+	-	-	-	-	Fail
Cinnamon-star anise	600,000	0	>1,100	-	-	-	-	-	Fail

Table 1 (cont)

Herbal medicine sample[a]	TAMC (CFU/g)	TYMC (CFU/g)	MPN (/100 ml)	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella* spp.	*Clostridium* spp.	Quality standard
Powder (n = 8)									
Yahom-1	84,000	0	35	-	-	-	-	-	Fail
Yahom-2	300,000	0	>1,100	+	-	-	-	-	Fail
Yahom-3	333,000	0	>1,100	+	-	-	-	-	Fail
Yahom-4	24,000	2	>1,100	-	-	+	-	-	Fail
Plai-*Zingiber cassumunar*	150,000	0	>1,100	-	-	-	-	-	Fail
Turmeric-1	600,000	0	>1,100	+	-	-	-	-	Fail
Thai herbs	1,050,000	0	>1,100	-	-	-	-	+	Fail
Yahom-5[b]	780,000	20	240	-	-	-	-	+	Fail

[a]Scientific name (italics) or Thai name if the sample has many ingredients; [b]Product regulated by the Ministry of Public Health Thailand
+ refers to positive bacterial culture while – refers to negative bacterial culture.
CFU: colony forming unit; g: gram; ml: milliliter; MPN: most probable number of coliform bacteria; TAMC: total aerobic microbial counts; TYMC: total yeast and mold counts

Table 2

Antibiogram and carriage of resistance genes

Bacterial isolate	Antibiogram		Resistance gene		
	Susceptible	Resistant	*bla*$_{CTX-M}$	*bla*$_{TEM}$	*bla*$_{SHV}$
Escherichia coli 04*	AK, CRO, CXM, FOX, FEP, G, IMP	AP, AUG, CIP, SXT	-	+	-
E. coli 07	AK, AUG, CIP, CRO, CXM, FEP, FOX, G, IMP, SXT	AP	-	+	-
E. coli 08	AK, AUG, CIP, CRO, CXM, FEP, FOX, G, IMP, SXT	AP	-	+	-
E. coli 13*	AK, CRO, CXM, FOX, FEP, G, IMP	AP, AUG, CIP, SXT	-	+	-
E. coli 20	AK, AUG, CIP, CRO, CXM, FEP, FOX, G, IMP, SXT	AP	-	+	-
E. coli 22	AK, FOX, G, IMP, SXT	AP, AUG, CIP, CRO, CXM, FEP	+	+	-
E. coli 33	AK, AUG, CIP, CRO, CXM, FEP, FOX, G, IMP, SXT	AP	-	+	-
E. coli 34	AK, FOX, G, IMP, SXT	AP, AUG, CIP, CRO, CXM, FEP	+	+	-
Pseudomonas aeruginosa 09	AK, CAZ, FEP, G, IMP, NET, TZP	-	-	+	-
P. aeruginosa 11	AK, CAZ, FEP, G, IMP, NET, TZP	-	-	+	-

*Multidrug resistance bacterium (resistance ≥3 classes of antibiotics) (Seng *et al*, 2018).
+ present; - absent.
E. coli and *P. aeruginosa* were tested against 11 and 7 antibiotics respectively (CLSI, 2021).
AK: amikacin (30 μg); AP: ampicillin (10 μg); AUG: amoxicillin/clavulanate (20/10 μg); CAZ: ceftazidime (30 μg); CIP: ciprofloxacin (5 μg); CRO: ceftriaxone (30 μg); CXM: cefuroxime (30 μg); FEP: cefepime (30 μg); FOX: cefoxitin (30 μg); G: gentamicin (10 μg); IMP: imipenem (10 μg); NET: netilmicin (30 μg); SXT:, trimethoprim/sulfamethoxazole (1.25/23.75 μg); TZP: piperacillin/tazobactam (100/10 μg)

(2nd generation cephalosporin), ceftriaxone (3rd generation cephalosporin) and cefepime (4th generation cephalosporin)] while no isolates carried *bla*$_{SHV}$ gene.

One sample of powder herbal medicine (from Nonthaburi Province) tested positive for steroid using an immunochromatographic method, and subsequently was identified as dexamethasone by the reference laboratory, Narcotics Control Division, Ministry of Public Health Thailand. Other adulterants found were paracetamol and diclofenac (a non-steroid anti-inflammatory drug). Additionally, in 32/59 random selected samples, two heavy metals, cadmium and lead, were detected but at levels well below the permissible limits (Table 3).

DISCUSSION

All samples in this study were primarily herbal medicines available in Thailand. Although herbal medicine is traditionally considered safe and is used extensively without prescriptions, reports of health issues linked to their use cannot be neglected (WHO, 2019; Kalumbi *et al*, 2020; Mautsoe *et al*, 2021). A lack of detection of contaminants, such as microbes, steroids and heavy metals, in herbal medicine, by providers can affect the health of users. This results in the trade of herbal medicines that patently are unsafe for consumption (Zheng *et al*, 2017; de Sousa Lima *et al*, 2020).

The study showed that more half of herbal medicinal samples collected

Table 3

Heavy metals in herbal medicine (n = 32)

Heavy metal	Range (mg/kg)	Mean ± SD (mg/kg)	Quality standard*
Lead	0.003 - 0.617	0.140 ± 0.009	Pass
Cadmium	0.003 - 0.264	0.038 ± 0.025	Pass

*All samples are within limit: cadmium and lead <0.3 and <10 mg/kg respectively (DMSC-MOPH, 2017).

mg/kg: milligram/kilogram; SD: standard deviation from Bangkok and four provinces failed the standards for TAMC and TYMC levels set by the Ministry of Public Health Thailand (MOPH, 2021). *Clostridium* spp constituted the highest proportion (19%) of pathogenic bacterial contamination, similar to that (18.64%) in an earlier report (Rangsipanuratn *et al*, 2017). The high fraction of bacterial contamination in herbal medicinal products sold in Thailand indicates poor sanitary practices in the preparation and packaging of this merchandise.

In Malawi, 20/29 samples of herbal medicine are contaminated with bacteria, 75% being coliform bacteria, 93% of which exceed the WHO recommended regulatory limit of $<10^3$ CFU/g (WHO, 2011; Kalumbi *et al*, 2020). The WHO recommended regulatory limit is the same as Thai Pharmacopeia standard used in the current study (DMSC-MOPH, 2017).

There were few fungi contaminations detected in the current work but they were not identified as to their genus and species. In China, in 45 samples of herbal medicine, 126 fungal isolates were obtained, with *Aspergillus* and *Penicillium* as the most predominant genera based on morphology and molecular analysis (Zheng *et al*, 2017). In Brazil (Bugno *et al*, 2006) and India (Moorthy *et al*, 2010) *Aspergillus* and *Penicillium* also were reported as the most predominant genera contaminating herbal medicine samples.

In Thailand, information on antibiotic resistant bacteria contaminating herbal medicine products is scarce. In the current study, bla_{TEM} gene was present in all *E. coli* (n = 8) and *P. aeruginosa* (n = 2) isolates contaminating the samples, a disconcerting discovery because it is the most widespread extended-spectrum β-lactamase gene carried by bacteria infecting humans (Zeynudin *et al*, 2018; Rohit *et al*, 2019). Two *E. coli* isolates in addition carried $bla_{\text{CTX-M}}$ gene that confers resistance to 2nd, 3rd and 4th generation cephalosporins (Zeynudin *et al*, 2018). Fortunately, these two *E. coli* isolates were different from the two multidrug resistant isolates, which were resistant to ampicillin, fluoroquinolone and sulfa drugs. A systematic review and meta-analysis in Africa reported an 86.51% prevalence of bacterial resistance in at least one herbal medicine product, with *E. coli* the most prevalent multidrug-resistant bacterium

(Walusansa *et al*, 2022).

There is zero tolerance for the presence of steroids in traditional medicinal herbal products, given their detrimental effects on human health if not prescribed by physicians for treatment of specific syndromes (Fung and Linn, 2017). In the current study, only dexamethasone was detected in a single (powder) herbal sample, but its level was not determined, but should be carried out. It will also be necessary to discover the source of this steroid contamination and in the meanwhile, the relevant authority should be alerted to remove this product from the marketplace.

Heavy metals contamination is another source of potential hazards to consumers of traditional herbal medicine, which is considered safe. As heavy metals are normal constituents of plants, WHO and (often) authorities in the countries producing herbal medicines have established permissible levels of heavy metals in such products. The requirements set by Thai Pharmacopoeia (DMSC-MOPH, 2017) are the same as those of WHO (2011). In the current study, the levels of cadmium and lead in all 59 herbal samples were much below the permissible levels, and presumably, were the normal compositions of the plant parts used in the preparations. Heavy metals in herbal medicinal products in Kumasi, Ghana, are lead (0.44-0.89 mg/kg) which is within legal limit, and cadmium (0.44-0.89 mg/kg), which is not (Nkansah *et al*, 2016). In China, the highest lead content in plants used in traditional medicine is 381.27 mg/kg of *Platcycladi cacumen* detected in Xingtai Hebei Province (Chen *et al*, 2021).

In conclusion, the study of microbial, steroid and heavy contaminations of herbal products (*n* = 59) obtained in Bangkok and four provinces showed that more than half of the samples failed the required limit of total microbe, yeast and mold counts, but passed the safety requirements for levels of heavy metals (cadmium and lead) and only one sample contained a steroid (dexamethasone). All *E. coli* (*n* = 8) and *P. aeruginosa* (*n* = 2) isolates carried bla_{TEM} gene. A quarter of *E. coli* isolates also carried $bla_{\text{CTX-M}}$ gene and another quarter were multidrug resistant. As the safety of herbal medicine is an important issue related to human health and has become an important factor restricting the export of herbal medicinal products, the results presented in the current study provide baseline data that should assist and help guide measures by manufacturers and government agencies to improve the quality and safety of traditional herbal medicinal commodities in the country for both local consumption and export.

ACKNOWLEDGEMENTS

This work was supported by Thailand Science Research and Innovation, Fundamental Fund Year 2022, research grant no. 25650001121791.

CONFLICTS OF INTERST DISCLOSURE

The authors declare no conflicts of interest.

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