Jurnal Sains Kesihatan Malaysia **15**(1) 2017: 7-17
DOI : http://dx.doi.org./10.17576/JSKM-2017-1501-02

Artikel Asli/*Original Articles*

Commercial Herbal Slimming Products: Evaluation of Heavy Metals and Microorganism Contamination at Different Batch Production

(Produk Pelangsingan Badan Berasaskan Herba Komersil: Penilaian Pencemaran Logam Berat dan Mikroorganisma pada Kelompok Pengeluaran Berbeza)

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ABSTRACT

Herbal-based slimming products which are consumed orally may be contaminated with heavy metals as well as microorganisms. This study aimed to evaluate the safety level of these slimming products by determining heavy metals and microbial contamination in different batch production. Six different brands of herbal-based slimming products (A, B, C, G, H and I) with three different batch productions (1, 2 and 3) were investigated (n =18). Five heavy metals Arsenic, Cadmium, Chromium, Copper and Zinc were determined using an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The presence of microorganisms was determined by total aerobic count and the bacteria were identified. The samples' moisture content was determined by calculating the percentage of water loss after drying process. All batches of samples A and B had high content of zinc, over the permissible level of 5ppm while, 6 samples contained Chromium above the permissible level (1.5 ppm). All 3 batches of sample A presented with the highest total daily intake of heavy metals. Bacteria were present in all the samples tested with the highest numbers in samples G, H and A followed by B, I and C. The highest number of fungi was found in product A while product I was free from fungal contamination. Aspergillus spp. *was the predominant fungus present in the samples. There was a weak correlation between moisture content and bacteria (r = 0.087) and fungal (r = 0.253) presence in the samples. As some herbal slimming products contain heavy metals as well as microorganisms, consumers need to be more vigilant and discerning when selecting products to be consumed.*

Keywords: Herbal-based slimming products; heavy metals; bacteria; fungi; Inductively-coupled plasma-mass spectrometry (ICP-MS)

ABSTRAK

Produk pelangsingan berasaskan herba yang diambil secara oral boleh dicemari logam berat dan juga mikroorganisma. Tujuan kajian ini adalah untuk menilai tahap keselamatan produk pelangsingan ini dengan penentuan tahap pencemaran logam berat dan mikroorganisma di dalam sampel pada kelompok pengeluaran yang berbeza. Sebanyak enam jenama produk pelangsingan berasaskan herba berbeza (A, B, C, G, H dan I) dengan kelompok pengeluaran berbeza (1, 2 dan 3) dikaji (n = 18). Penentuan lima logam berat Arsenik, Kadmium, Kromium, Kuprum dan Zink ditentukan dengan menggunakan Plasma Gandingan Induktif – Spektrometri Jisim (ICP-MS). Kehadiran mikroorganisma dalam sampel ditentukan dengan mengira kiraan jumlah mikroorganisma aerobik dan pengenalpastian pencilan dilakukan. Kandungan kelembapan sampel ditentukan melalui pengiran peratus kehilangan air di dalam sampel selepas proses pengeringan. Kesemua kelompok pengeluaran sampel A dan B mengandungi kandungan Zink yang tinggi dan melebihi had yang dibenarkan iaitu 5 ppm, manakala kesemua sampel didapati mengandungi kandungan Kromium melebih had yang dibenarkan iaitu 1.5 ppm. Sampel A menunjukkan kandungan logam berat-jumlah ambilan harian tertinggi secara konsisten bagi semua kelompok pengeluaran. Didapati bakteria terdapat pada kesemua sampel G, H dan A diikuti sampel B, I dan C. Bilangan fungi tertinggi didapati dalam sampel A, manakala sampel I bebas daripada kontaminasi fungi. Aspergillus spp. m*erupakan jenis fungi dominan yang dijumpai dalam sampel yang dikaji. Terdapat kolerasi lemah di antara kandungan kelembapan dengan pertumbuhan bakteria (r = 0.087) dan fungi (r = 0.253) di dalam sampel. Melihat tahap pencemaran logam berat dan mikroorganisma di dalam produk yang dikaji, pengguna perlu lebih berhati-hati dan mengambil langkah berjaga-jaga dalam memilih produk yang diambil.*

Kata kunci: Produk pelangsingan berasaskan herba, logam berat; bakteria; fungus; Plasma Gandingan Induktif – Spektrometri Jisim (ICP-MS)

INTRODUCTION

The World Health Organization (WHO) had reported the rising prevalence of overweightness and obesity amongst Malaysians (WHO 2012). Malaysian women were more overweight at 46%, compared to men at 29%. Compared to the other ASEAN countries listed in the WHO report, Malaysia had the highest obesity prevalence. The overweight and obesity prevalence in the Malaysian population increased by 29.1% and 14.0%, respectively, from 1996 to 2009 (Khambalia & Seen 2010).

The rise of overweight and obesity problems among Malaysians had encouraged the influx of slimming products in the market as well as usage among consumers. In Malaysia, the herbal product market is currently experiencing enormous growth, with the combination of herbal therapy into modern medical practice by many health professionals (Ibrahim 2006). As reported by Salleh (1998), a total of RM1.2 billion a year was spend to import herbal products.

Herbal-based slimming products are registered as natural remedies or traditional medicines. Herbal-based medicines are normally derived from herbal plant sources which include the dried herbs or any other plant parts such as the leaves, roots, flowers, or seeds. Herbal medicines are also popular among the traditional medical practitioners. They are considered as health products which contain herbal ingredients as well as have beneficial factors for certain treatments (National Pharmaceutical Control Bureau 2010).

Normally, herbal-based slimming products can be easily obtained in the pharmacies as 'Over the Counter Product,' meaning the users can easily purchase them without requiring any doctor's prescription and restriction (WHO 2012). These products usually were made from crude herbs or herbal extracts which are claim can reduce the body weight. Protein from soy bean (Hikagi et al. 2006) and phosphatidylcholine in green tea extract (Juhel et al. 2000) had been used in slimming product as lipase inhibitor. Meanwhile, in Indonesia herbs such as *Zingiber cassumunar Roxb*., *Guazuma ulmifolis Lamk* and *Murraya paniculata* had been used actively as anti-obesity drugs (Iswantini et al. 2011).

To ensure that all products are safe to be consumed, Good Manufacturing Practices (GMP) should been implemented by the manufacturers. Various product testing should be carried out such as quality analysis, weight uniformity test and test for the limits of heavy metal and microorganisms' contamination levels before reaching to the customers. Even though herbs are natural and considered harmless, side effects have been reported either due to herbal-plants itself or contaminants such as heavy metals, microorganisms, adulterants and synthetic materials (Ernest 1998; Bent & Ko 2004; De Smet 2004). The quality of herbal based products depends on the source of raw materials and productions process. Environmental conditions may contribute to the contamination of the products and may have occurred during the planting, harvesting of raw materials as well as during production process (WHO 2005).

Heavy metals contamination such as Fe, Cu, Zn, As, Pb and Cd have been found in some Chinese herbal-based medicines (Liang et al. 1998). Our previous study also showed the same findings where seven out of ten samples studied, contained heavy metals such as As, Cd, Cr, Cu and Zn, as well as bacterial contamination (Zin et al. 2014). Microbial contamination was also reported in herbal products in Nigeria, *E. coli* was found in 10 out of 21 tested samples while, 7 samples contained *Salmonella spp.*, 15 samples contained *S. aureus* and 12 others were contaminated with fungi (Adenike et al. 2007).

Batch production numbers are the combination of numbers or letters or both of which are very specific and can be used to identify a certain period and particular groups of product manufactured (Glossary European Commission 2001). Validation of product assessments according to their particular groups is important to ensure consistency of the quality and safety of the in production, as well as to ensure the products were complying with the regulations. Hence, differences between batch productions need to be assessed to confirm repetition of heavy metals and microorganism contaminations, if any. Therefore, this study was carried out to determine the presence of heavy metals and microorganisms in the herbal-based slimming products in different batch of productions.

MATERIAL AND METHODS

SAMPLE COLLECTION

The products tested were the same as in our previous study (Zin et al. (2014). Six products labeled A, B, C, G, H and I at different batch production (n = 18) were purchased from traditional herbal medicine shops and health products around Kuala Lumpur (Table 1).

TABLE 1. Details of six different brands of herbal slimming products. Selected samples were based on a previous study by Zin et al. (2014)

Sample*	Product Form	Daily dose (g × package/capsule/tablet)	Manufacturing country
A	Powder	7.00 × 1	Indonesia
B	Capsule	0.50 × 3	Indonesia
C	Capsule	0.35 × 2	Indonesia
G	Capsule	0.50 × (4-5)	Malaysia
H	Capsule	0.50 × (4-5)	Malaysia
I	Tablet	0.75 × 2	Indonesia

DETERMINATION OF HEAVY METAL CONTENT AT DIFFERENT BATCH NUMBER

The samples were digested using the modified EPA 200.3 method (EPA 1991) previously used (Zin et al. 2014). All the digested samples' solutions were filtered with a syringe filter (0.45 µm) and analyzed for the presence of heavy metals (As, Cd, Cr, Cu and Zn) using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) ELAN 9000 (PerkinElmer, Sciex USA) with the operating conditions stated in Table 2.

TABLE 2. Operating conditions for ELAN 9000 ICP-Mass Spectrometer

ICP-MS hardware	Operation Condition
RF Power	1000 W
Sampler Diameter	1.1 mm
Sample skimmer cone	Ni
Nebulizer	Cross-flow (Mainhard)
Peristaltic pump	1 ml/min
Argon Flow rate plasma	15 L/min
Nebulizer flow	0.9 L/min
Spray chamber	Scott double pass

DETERMINATION OF MOISTURE CONTENT

Samples were dried in the oven until the consistent weight value was obtained. The moisture content was determined by calculating the percentage of weight loss cause by the drying process (Vikosen & Alade 2011).

DETERMINATION OF BACTERIAL PRESENCE

The presence of bacteria in the samples was determined by spread plate method. About 1.0 g sample was dissolved in 9 ml of phosphate buffered saline, serially diluted, plated onto Tripticase Soy Agar (TSA), incubated at 37°C for 24-48 hours and colonies formed were calculated as colony forming units (CFU/g). All samples were tested in triplicates.

IDENTIFICATION OF THE BACTERIA

Isolated bacteria were identified by macroscopic and microscopic observation, (Yap et al. (1999); Aldrige et al. (1978) routine biochemical tests and confirmed commercial by API 20E kit for Enterobacteriacae and other non-fastidious gram negative bacteria, API CHB 50 for *Bacillus* sp. and API STAPH for *Staphylococci* and micrococci (BioMerieux).

DETERMINATION OF FUNGAL PRESENCE

About 1.0 g of each sample was mixed with 9.0 ml of 0.9% (wt/v) normal saline (NaCl) solution. Serial dilutions (in 0.1% peptone) were carried out and plated on Potato Dextrose Agar (PDA) plates containing 0.01% chloramphenicol (0.1 ml/plate) to inhibit bacteria. Samples were tested in triplicates and plates were incubated for 14 days at 27°C.

IDENTIFICATION OF FUNGI

Fungal isolates were identified by macroscopic and microscopic observations. Macroscopic observations included colony morphology such as shape, texture and color. Microscopic observations determined the special features found on the fungi as described by Larone (1995).

RESULTS

DETERMINATION OF HEAVY METAL CONTENT AT DIFFERENT BATCH NUMBER

From this study, Arsenic (As), Cadmium (Cd), Chromium (Cr), Zinc (Zn) and Copper (Cu) were detected consistently in all samples. Arsenic was present in all samples tested with the second batch production showing the highest (Figure 1). Sample H and G (batch 2) showed As content of 1336.7 ± 0.22 ppb and 1215.30 ± 0.18 ppb, respectively. However, it was still under the permissible limit of 5000 ppb (WHO 1992). However more stringent monitoring program should be taken as there are some batch production showed the increment of As content over the permissible values.

For Cadmium (Cd), sample A and B from all batch productions showed a higher content compared to the other samples (Figure 2). However none of the samples tested had exceeded the permissible limit of 300 ppb (WHO 1999). The determination of Chromium (Cr) content in 18 samples revealed that 5 samples exceeded the standard limit of 1500 ppb (DCA 2012) (Figure 3). Sample G from batch 1 had the highest content of Cr which was 2462.60 ± 0.22 ppb and sample C from batch 1 showed the lowest amount at 458 ± 0.08 ppb. However, from Cr content pattern in sample C showed an increment in all three batches and were exceeded the permissible limits.

Products A and B showed high content of Copper (Cu) particularly in batch 1 sample A with 3539 ± 0.02 ppb. Nevertheless, the value was still under the standard limit allowed by DCA (2012) at 10 000 ppb (Figure 4). Meanwhile for Zinc (Zn), a high content of Zn was detected in all batch production samples of product A ranging from 18828.70 to 24140.10 ppb of 1g of samples (Figure 5). This amount is markedly higher than the permissible level of 5000 ppb (DCA 2012).

Total Daily Intake (TDI) of heavy metals (ng/day) for each of the samples are shown in Table 3. The value was based on the manufacturers' recommended daily dose for the six products. If a consumer consumed any of the tested slimming product samples, the TDI for each heavy metals as followed; Cr ranged between 320.0 to 14073.0 ng/day, Cu ranged between 189.00 to 24 773.00 ng/day, Zn ranged between 2 013.00 to 168 981.00 day and Cd ranged between 6.00 to 494.00 ng/day. Among these six

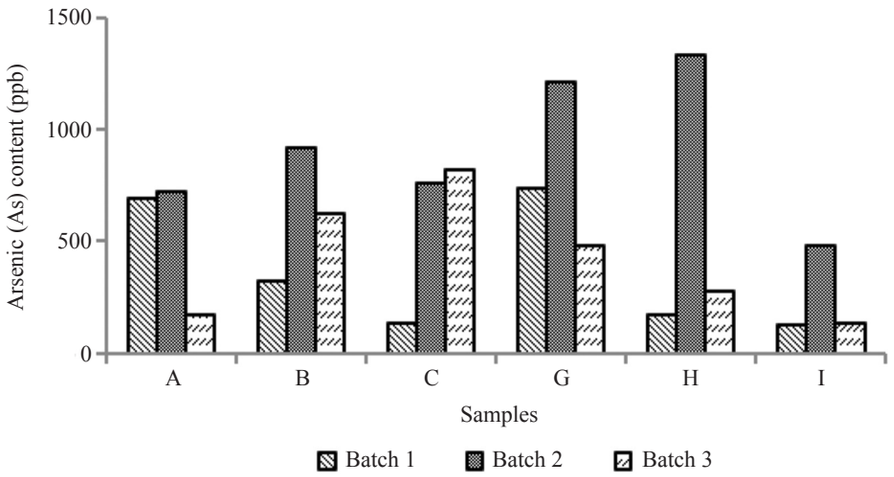

FIGURE 1. Arsenic (As) content (ppb) in 1g samples at different batch production (*As permissible value = 5 000 ppb (5 ppm) (WHO, 1992)*)

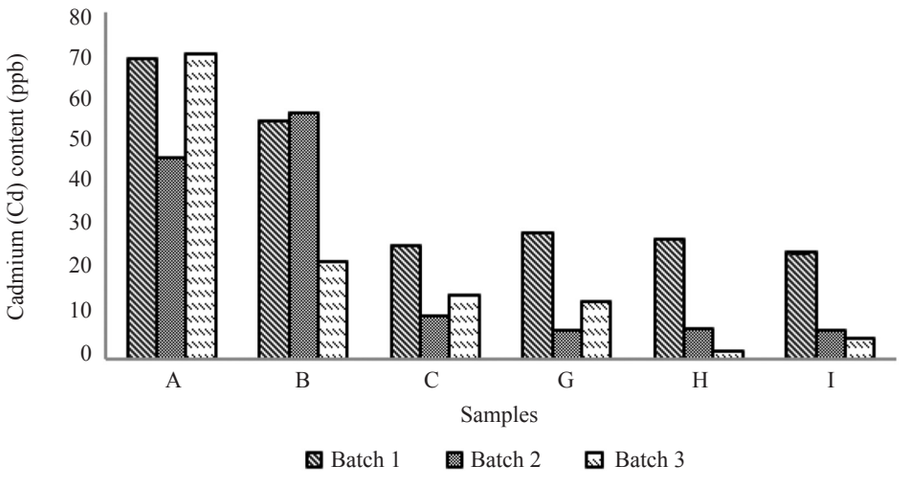

FIGURE 2. Cadmium (Cd) content (ppb) in 1g samples at different batch production (*Cd permissible value = 300 ppb (WHO 1999)*)

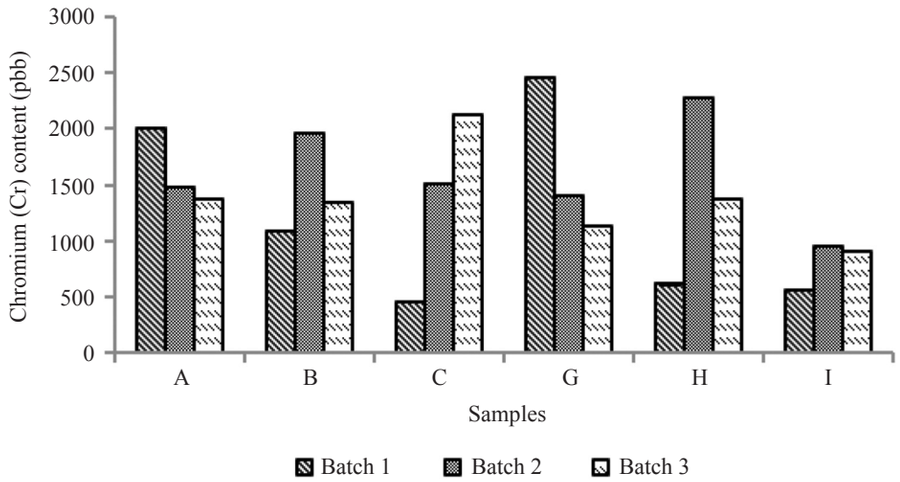

FIGURE 3. Chromium (Cr) content (ppb) in 1g samples at different batch production (*Cr permissible value = 1 500 ppb (1.5 ppm) (DCA 2012)*)

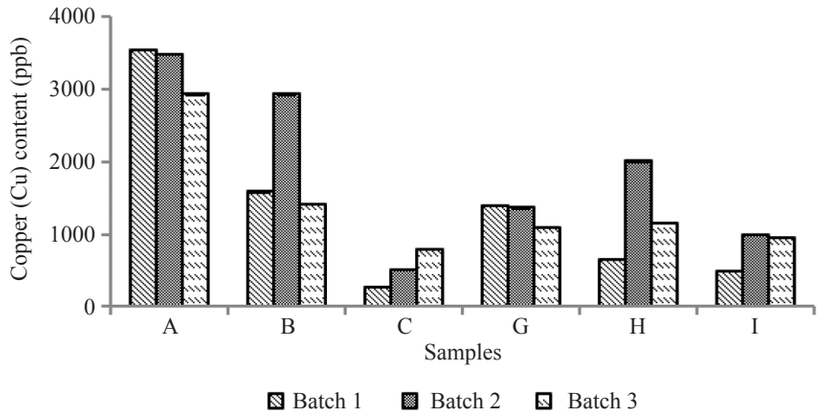

FIGURE 4. Copper (Cu) content (ppb) in 1g samples at different batch production (*Cu permissible value 10 000 ppb (10 ppm) (DCA 2012)*)

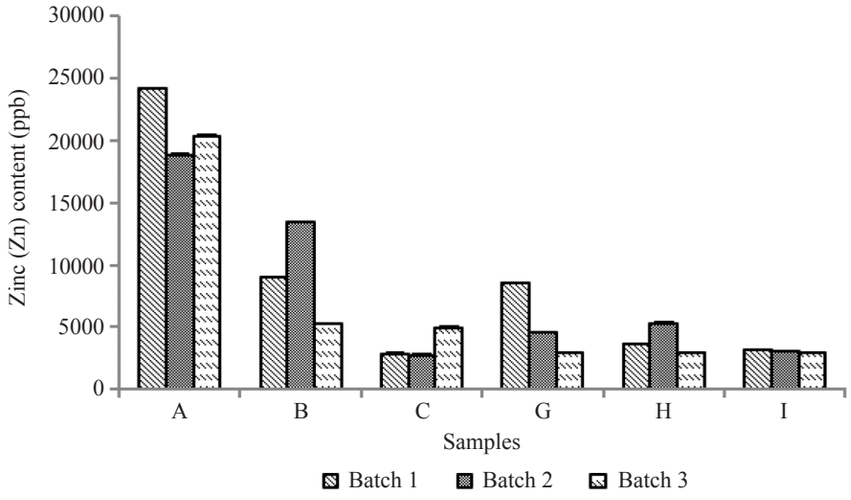

FIGURE 5. Zinc (Zn) content (ppb) in 1g samples at different batch production (*Zn permissible value 5 000 ppb (5 ppm) (DCA 2012)*)

TABLE 3. Daily Consumption for Heavy Metals (ng/day) for all samples tested

Samples	Batch	As	Cd	Cr	Cu	Zn
A	1	4828.60	485.10	14 073.00	24 773.00	168 981.00
	2	5041.40	324.10	10 364.00	24 347.40	131 801.00
	3	1206.00	494.00	9600.00	20 496.00	142 481.00
B	1	489.00	83.00	1626.00	2375.00	13 480.00
	2	1382.00	85.20	2948.00	4391.00	20 087.00
	3	940.00	33.60	2009.00	2124.00	7858.00
C	1	93.90	18.30	321.20	189.10	2015.30
	2	532.10	6.90	1056.60	359.50	1941.80
	3	576.40	10.30	1490.20	548.20	3493.30
G	1	2201.70	7387.80	87.60	4199.10	25 774.20
	2	3645.90	4222.50	19.50	4089.00	13 594.20
	3	1446.00	3415.20	39.60	3285.60	8841.90
H	1	501.60	83.40	1847.40	1959.00	11 106.60
	2	4010.10	21.00	6853.20	6015.30	15 999.90
	3	822.90	5.40	4134.00	3428.40	8841.00
I	1	183.60	36.80	836.10	747.80	4761.90
	2	722.70	9.90	1440.00	1480.40	4522.50
	3	195.20	7.20	1359.90	1414.10	4439.00

** Permissible limit TDI value of heavy metals contained in herbal products*
Cr- 20 000 ng/day; Cu- 150 000 ng/day; Zn-20 000 ng/day; As-10 000 ng/day; Cd- 7 000 ng/day

products; product A samples showed highest content and mean intake for all five heavy metals.

DETERMINATION OF BACTERIA AND FUNGI PRESENCE

The number of bacteria and fungi present in each product sample for the different production batches are shown in Table 4. Product G samples showed the highest contamination of bacteria ranging from 3.85 x 10^{11} to 4.5 × 10^{11} CFU/g while product C samples had the lowest bacteria counts ranging from 1.7 × 10^{10} to 2 × 10^{10} CFU/g. A total of 16 different fungal isolates were obtained from various samples with product A samples showed the highest contamination with fungi while product I sample showed absence of fungi.

TABLE 4. Colony formation units (cfu/g) and total number of different strain of fungi presence in samples at different batch production

Samples	Batch 1		Batch 2		Batch 3	
	Bacteria (cfu/g)	Fungi	Bacteria (cfu/g)	Fungi	Bacteria (cfu/g)	Fungi
A	3.43×10^{11}	5	1.66×10^{11}	1	3.42×10^{11}	-
B	1.16×10^{11}	-	9.00×10^{9}	1	4.50×10^{11}	-
C	1.70×10^{10}	2	2.00×10^{10}	-	1.9×10^{10}	-
G	4.13×10^{11}	3	3.85×10^{11}	-	4.50×10^{11}	-
H	4.50×10^{11}	1	4.50×10^{11}	-	1.38×10^{10}	3
I	4.50×10^{10}	-	5.80×10^{10}	-	1.1 10^{11}	-

** (-) Indicates no presence of fungi*

IDENTIFICATION OF BACTERIA AND FUNGI

A total of 46 different bacterial strains were determined from all product samples. Twenty seven (27) of the isolates were Gram negative bacteria and nineteen were Gram positive bacteria. For the Gram negative bacteria, eight of them were coccus-shaped cell, thirteen had coccobacilli-shaped cell and six isolate with rod-shaped. Out of nineteen Gram positive isolates, nine had coccus-shape, 5 with rod-shape and 5 with spiraling-shaped. After macroscopic observation and routine biochemical tests, only 13 isolates with unique cell and colony characteristic were identified using commercial kits. The isolates were identified as *Pseudomonas aeruginosa*, *Neisseria meningitidis*, *Capnocytophaga* spp., *Corynebacterium* spp., *Brucella* spp., *Escherichia coli*, *Bacillus* spp., *Yersinia* spp., *Enterobacteriaceae* spp., *Staphylococcus aureus* and *Streptococcus* spp. (Table 5). For the fungal identification, five fungal isolates from product A samples were identified as *Penicillium* sp., *Aspergillus* spp and *Rhizomucor*. Similar strains of fungi (*Penicillium* sp. and *Aspergillus* spp) were also found in brand G, B and C samples (Table 5).

TABLE 5. Identification of microorganism's presence in samples tested

Product	Isolates identification	
	Bacteria	Fungi
A	• *Pseudomonas aeruginosa* • *Neisseria meningitidis* • *Corynebacterium spp.* • *Brucella spp.* • *Capnocytophaga spp.* • *Corynebacterium spp.* • *Brucella spp.*	• *Aspergillus fumigatus* • *Aspergillus glaucus* • *Aspergillus nidulans* • *Penicillium*
B	• *Escherichia coli* • *Bacillus spp.*	• *Aspergillus fumigatus*
C	Not determined	• *Aspergillus fumigatus* • *Aspergillus niger*
G	• *Enterobacteriaceae spp.* • *Neisseria meningitidis* • *Enterobacteriaceae spp.*	• *Aspergillus fumigatus* • *Aspergillus nidulans* • *Penicillium*
H	• *Brucella spp.* • *Yersinia spp.*	• *Aspergillus glaucus* • *Aspergillus fumigatus*
I	• *Escherichia coli,* • *Enterobacteriaceae spp.* • *Bacillus spp.* • *Staphylococcus aureus* • *Streptococcus spp.* • *Bacillus anthracis*	Not determined

DETERMINATION OF MOISTURE CONTENT

The moisture content varied in the product samples with products A and B containing more moisture while product C had the least moisture content (Figure 6). However, only a weak correlation (r = 0.087) was found between the moisture content and the bacterial presence. Similarly, fungal presence was not affected by the moisture of the sample (r = 0.253).

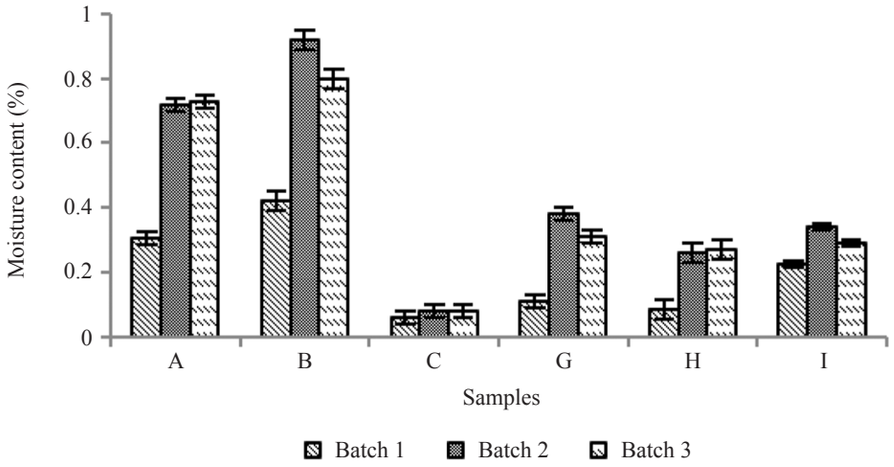

FIGURE 6. Moisture content (%) for the each samples brand at different batch production number

DISCUSSION

The two main issues of herbal-based products are their safety and efficacy which could be affected by the source and the quality of the raw materials (Rao and Kumar (2007). According to Ibrahim (2006), no analytical study has been carried out on herbal-based products in the local market to ensure all of them were good quality and safe to be consumed. Heavy metals and biological contamination were the major issues because they could affect consumers' long-term health (Khan et al. 2012). According to WHO (2007), chronic accumulation of trace metals due to long term consumption of medicinal plants may be harmful to the health of consumers. The excess exposure to the heavy metals may cause metabolic function interfere (Singh et al. 2011).

Generally, metals play a vital role as structural and functional components of protein and enzymes in cells. Metals such as Ca, Mg and Zn have been claimed as essential for human health, however less than 100 mg per day is required (Sharma et al. 2011). Metals such as Pb, Cd and Al have been identified as toxic and their content in medicine need to be controlled (WHO 1991). All the tested samples showed the presents of heavy metals and microorganisms consistent with our previous study on over 10 different selected brands (Zin et al. 2014). In this study, Cr and Zn content in the samples exceeded the permissible limit set by WHO (1999) and DCA (2012).

Zinc and Copper were the essential metals that needed for the physiological activity in the human body. In this study, Zn content was highest in all samples. At the trace level, Zn is an essential element, found in almost all body tissue (Muhammad et al. 2010). Although Zn is needed for enzyme catalytic activities, cell signaling, release of hormone and apoptosis (Troung-Tran et al. 2000; Muhammad et al. 2010), an excessive consumption of this element can lead to health problems such as stomach cramps, skin irritations, vomiting, nausea and anemia. (Lukaski et al. 2007; Saeed 2010). Major sources of Zn in the environment are due to its use in Zn batteries and furniture industries.

Another essential metal Cu, is needed to regulate various biological systems. It plays major role in the metabolism of iron and acts as cofactor in some enzymatic systems. Cu is required for normal biological activities of amino oxides and tyrosinase enzyme, for catalytic conversion of tyrosine to melanin, which protects the skin from dangerous radiations (Hashimi et al. 2007). Cu deficiency also may lead to the impairment of iron absorption and cause anemia (Soetan et al. 2010). However, excess Cu in the body may interfere gastrointestinal tract and result in nausea, vomiting and diarrhea (Pizarro et al. 1999).

Even though all samples contain low level of cadmium, the presence of it should be given appropriate attention because it is one of the human carcinogenic agents. Liver and kidney are the main target organs, in acute and chronic Cd exposure (IARC 1994). Due to the long biological half-life, excessive accumulation of Cd can damage kidney and bone function and poses a high risk for kidney cancer (Kolonel 1976).

Even though in this study the Cd content in all six samples was still under permissible limit, Cd was is well-known environmental toxin associated with an increased risk of cancer and cardiovascular disease in humans (Wu et al. 2003). Long-term exposure to this element may cause

hyperkeratosis or skin pigmentation changes (Jarup 2003) and at the same time may cause skin, lung, bladder and kidney cancer (WHO 2012).

Since there are permissible limits prescribed for chromium (Cr), copper (Cu), zinc (Zn) and cadmium (Cd) in Malaysia, therefore the quantity of daily consumption of each heavy metal was determine based on the manufacturers' recommended daily consumption dose and compared with Tolerance Daily intake (TDI) recommended by Health Canada (2004). Findings from this study showed TDI values for most of the heavy metals in this study were under the permissible standard limit except for Zn in product A samples. According to DCA (2012), the maximum consumption of Zn for a person was 20 000 ng/day, however this value is lower than that recommended by the manufactures (>130 000 ng/day). Consumers of this brand may have adverse health effects due to excessive Zn consumption. According to the Linus Pauling Institute (LPI), a single dose of 225 to 450 mg of Zn usually induces vomiting while milder gastrointestinal distress was reported at doses of 50 to 150 mg/day of consumed.

It's the manufacturers' responsibility to ensure all their products safety and free from other contaminants (WHO 2006). The differences of heavy metals content in different batches each product, may due to lack of Good Manufacturing Practice (GMP) knowledge among the manufacturers. This guideline helps manufactures' to reduce the contamination; it may help manufactures' improve their practices start from sampling to productions of herbal products.

The batches contamination by heavy metals can be reduce by the manufacturers' Quality Assurance (QA) monitoring programs. By this program the detection of heavy metals in each batch product can be avoided in the early stage of production. The inconsistency of heavy metals content in different batches of products also may be due to the personnel self-hygiene. Personnel's entrusted the whole products manufactured and required to have high degree of personal hygiene. Personnel must have adequate knowledge and training program in maintaining the appropriate hygiene level.

Contamination of herbal products by heavy metals may be due to many factors such as the herbal sources itself or may occur during the manufacturing process or exposure to heavy metals in the environment during transportation (Zhang et al. 2012). In this study, we found that presence of heavy metals was consistent for each batch products. This indicates the possibility of contamination from herbal sources or during product manufacturing. Heavy metals contamination of herbaceous plants may be due contaminated plantation areas. Herbaceous plant grown within city areas were exposed to pollution from manufacturing activities, smoke from motor vehicles and other sources of pollution (Mosihuzzaman & Choudry 2008); (WHO 2007). Plants absorb heavy metals in contaminated water through roots or other exposed plant parts which is not equally distributed over the entire plant (Tokahuglu 2012). Therefore the level of heavy metal contamination in each herbal product would differ, depending on which plant part was used. The process of harvesting, drying, storage, handling and the soil influence the quality of raw material which in turns affects the entire quality of the herbal preparation.

According to European Pharmacopoeia (2007), for products that are required to be mixed with boiling water, the maximum number of bacterial should not exceed 10^7 CFU/g sample while for the directly consumed product, the bacteria limit is 10^5 CFU/g. In this study, all the samples contained high levels of bacteria, above the European Pharmacopoeia limit, which included pathogenic Gram negative bacteria (*Salmonella* and *E. coli*). This result supported our previous finding that products samples G and H contained the most bacterial contamination in all three batches (Zin et al. (2014). A Nigerian study, also found bacterial contamination in 56% of herbal product samples with a highest total aerobic count of larger than $5' 10^7$ CFU/g, exceeding European Pharmacopoeia limit (Abba et al. (2009).

Another study found 74 bacterial and 55 fungal strains including *Bacillus*, *Staphylococcus*, *Klebsiella, Listeria, Micrococcus, Cornybacterium, Proteus, Escherichia coli, Streptococcus, Acinetobacter*, *Citrobacter, Lactobacillus a*nd *Serratia* in 17 herbal drug preparations (Esimone et al. 2007). *E. coli* is an indicator of fecal contamination indicating possible presence of disease-causing organisms (APHA 1992; Jay 1997). The presence of pathogenic bacteria in the samples may be due to the non-hygienic processing methods or the equipment, raw materials used during the production and personnel. The bacteria could be transmitted during handling of the raw materials during processing. Herbal raw ingredients with high starch content may be prone to increased microbial growth.

In general, bacterial contamination can cause poisoning to the consumers, microorganisms such as *Bacillus* spp., *E. coli* and *Staphylococcus aureus* were the common bacteria identified as the cause for food poisoning cases. The possibility to be infected by these pathogenic bacterial was very high since most of the products were taken orally. Addition of water at temperatures below 70°C to the products will influence the bacterial growth, since the pathogen is not killed at this temperature (James et al. 2007).

Apart from bacterial contamination, the results also showed the presence of fungi in some of the products. All samples except product I were contaminated with *Aspergillus* spp and *Penicillium* sp. Fungi are widespread in the environment and are common natural contaminants of herbs. Some fungal species can produce toxic metabolites (mycotoxins) under favorable conditions. In Brazil, 91 samples of herbal plants evaluated for fungal contamination showed that 89.9% of the isolates were *Aspergillus* and *Penicillum* strain which are potentially mycotoxigenic (Bugno et al. 2006). In Nigeria, the evaluation of herbal products in various areas showed that most of the samples

were contaminated by *Aspergillu*s and *Penicillum*, however *Mucor* spp., *Candida* spp., *Trichosporium* spp. were also found in the samples (Anyanwu 2010).

The presence of bacteria and fungi in the sample may be influenced by product formulation whether in powder, capsule or liquid form. In this study, most of the fungi were isolated from samples that were in powder form. A similar finding was reported by Gautam et al. (2008); which proved samples produced in powder form contained more bacteria compared to tablet form. Products in powder form are more susceptible to the environment, resulting in higher contamination compared to products produced in other forms.

In our study moisture in the herbal products was not a major factor for microbial contamination as a weak correlation was observed between moisture content and presence of microorganisms. This differs from other findings (Abba et al. 2009), where there were no bacterial counts in herbal preparations at very low moisture content of less than 6% while high bacteria counts were observed at high moisture content.

Theoretically, the moisture content is one of the abiotic factors that influences the growth of microorganisms, particularly bacteria. However, fungi, especially *Aspergillus* spp. can survive at low water content. This is borne out by a study which found no significant correlation between the levels of water content and the presence of fungi in the samples (Priyanka et al. 2008).

CONCLUSION

Consumers of herbal slimming products sold in Malaysia, need to be aware that these products may not comply with international quality and safety standards. To ensure the safety and quality of these products strict guidelines by local authorities must be developed.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Science Technology and Innovation (MOSTI) Malaysia under project code FRGS/1/2011/ST/UKM/02/1.

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Received: July 2015
Accepted for publication: July 2016