

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Commercial Herbal Slimming Products: Concern for the Presence of Heavy Metals and Bacteria

Noraziah Mohamad Zin, Yong Mei Chit and Nur Faizah Abu Bakar  
Programme of Biomedical Science, School of Diagnostic and Applied Health,  
Faculty of Health Sciences, Universiti Kebangsaan Malaysia,  
50300 Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia

**Abstract:** The increment of rate in obesity, the phenomenon of fat phobia as well as the increased use of herbal medicine had lead to the emergence of herbal slimming products. However, numerous bacteria and heavy metal contaminations are often found in herbal products due to irregular handling practices. Ten different brands of products (labeled as A-J) were investigated. Seven heavy metals content such as As, Cd, Pb, Co, Cr, Cu and Zn were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and bacterial presence was determined by counting the total aerobic count. The identification of isolates was carried out by macroscopic and microscopic observation, biochemical tests and confirmation using commercial kits of Microgen GN-ID A+B and API 20 E. The heavy metal contents in the samples were below the limit of the standard limitation by WHO and Health Canada. However, sample A contained the highest total daily intake of heavy metals. Total aerobic count was highest in sample H followed by G, A, B, C, F, D, E, I and J in which G and H exceeded the standard total aerobic count ( $10^5$  CFU  $g^{-1}$ ) as given by WHO. A total of nine isolates of *Bacillus* spp. and ten gram-negative bacteria were isolated in which *Bacillus cereus* and *Pseudomonas aeruginosa* were found in samples C and F, respectively. Considering the fact that the herbal slimming products contained low concentration of heavy metals and bacteria count, it should be consumed with caution.

**Key words:** Herbal slimming products, heavy metal, bacteria

### INTRODUCTION

The growing trend of obesity in the Malaysian population is steadily becoming a public health challenge (Geok, 2012). The second and third National Health and Morbidity Surveys in 1996 and 2006, respectively have stated a three-fold rise in obesity prevalence among adults, which were surging from 4.4 to 14% within ten years (Institute of Public Health, 2008). Based on the Malaysian Non-Communicable Disease Surveillance, the risk of obesity among Malaysian adults is higher among women and those with family history of illnesses, such as hypertension, diabetes and cardiovascular disease (Tan *et al.*, 2011).

The phenomenon of fat phobia is a serious problem, especially among the women since the desire of slender body appearance is much associated with self-satisfaction and one's social position (Izydorczyk, 2011). Nowadays, women prefer to choose slimming product rather than taking care of their diet or engaging in physical activity to lose their weight (Riji, 2006). Therefore, this has prompted the development of the slimming products in the market.

Herbal products are crude drug which are made from raw herbs or herb extracts with the mixture of phytochemicals and may contain single herbal ingredient or mixture small doses of multiple variation of herbal plant part (Jantan, 2006). Usually it comes to the market either in the form of powders, pills or capsules (Kosalec *et al.*, 2009). The notion that herb is safe because of its natural background and have long record of use in folk medicine have encouraged the emergence of herbal products (Kosalec *et al.*, 2009; Jantan, 2006). With the increasing of usage of traditional Asian medicine in developed countries, the slimming products based on herbs are being popular in the market (Ernst and White, 2000).

However, the safety of herbal products has become a major concern in public health with the growing popularity of consumer and market expansion globally (WHO, 2004). Lack of monitoring by the authorized organization may contribute the attributable the low quality of herbal products in the market (Kosalec *et al.*, 2009). The adulteration of herbal products with undeclared drugs, heavy metals, hazardous microbial metabolite (mycotoxins), radioactive particles and

**Corresponding Author:** Noraziah Mohamad Zin, Programme of Biomedical Science, School of Diagnostic and Applied Health, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia

pesticide can affect the quality of herbal products production (WHO, 2004; Ernst, 2002). In Malaysia, there are no strict analytical controls been carried out on herbal products for ensuring only high quality products are approved for the local market (Jantan, 2006). Herbal product contamination could happen in many ways such as from environment, during processing or transportation stage (Mosihuzzaman and Choudhary, 2008).

Heavy metals are widely distributed naturally throughout the earth with density more than 5 g cm<sup>-3</sup> and can be released into the environment through soil and water (Jarup, 2003). Heavy metals could be essential nutrient and harmful to the human even in low concentration. Heavy metals can be classified as potential toxic such as aluminium, arsenic, cadmium, lead and mercury, probably essential such as nickel, vanadium and cobalt and essential such as iron, manganese, ferum, copper, zinc and selenium (McIntyre, 2003). The essential metals can give toxic effect when it was taken excessively by the consumer (Uluozlu *et al.*, 2009). Exposure to Pb, Cd, Hg and As can be the major threats to human health (Athar and Vohora, 1995; Singh *et al.*, 2011).

Biological contamination refers to contamination of herbaceous plants by microorganisms such as bacteria, fungi (molds), viruses, protozoa, insects (eggs and larvae) and other organisms. The presence of pathogenic microorganisms in herbs might pose a risk to public health (Banerjee and Sarkar, 2003; ICMSF, 2005) and affects the quality of the products. *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes* and spore-forming microorganisms such as *Bacillus cereus* and *Clostridium perfringens* are those listed pathogenic microorganisms (Witkowska *et al.*, 2011) Enterobacteriaceae spp. and *Pseudomonas* spp. are the two groups of bacteria found commonly on the harvested plant surface and these can rise to the problem of damage and deterioration in the quality of food (Baylis, 2006). Thus, it is important to monitor the bacteria contamination in any herbal products.

Herbal medicine products are not really free from toxicity and can lead to other adverse effects (De Smet, 2004). Problem of heavy metal poisoning and bacterial contamination are a popular issues related to the safety of these products (WHO, 2004; Ernst, 2002). Implementation of quality standards on testing is very important for security and safety to consumers (Kosalec *et al.*, 2009). Thus, this study aimed to determine if the herbal slimming products were free of heavy metal and bacterial contamination for the safety of users and consumers.

## MATERIALS AND METHODS

**Sample collection:** A total of ten herbal slimming products of different brands were obtained from traditional herbal medicine shops and health products selling stores around Kuala Lumpur and were labeled as sample A to J. Details of these samples as listed in Table 1.

**Determination of heavy metal content:** All the samples were performed with pre-treatment by acid digestion based on modified standard procedures of EPA 200.3 (McDaniel, 1991). A total of 5 g sample was mixed with 10 mL of concentrated nitric acid and heated until the color changed to brown. Sample was cooled and then added with 10 mL of nitric acid. It was heated again until the solution turned brown. Then, sample was cooled and 2 mL of nitric acid was added and the process was repeated until the volume became 5 to 10 mL. Next, 2 mL of hydrogen peroxide was added to sample. The process of adding hydrogen peroxide solution, heating and cooling were repeated until the sample solution turned into a clear sight. Then, the sample was cooled and added with 2 mL of hydrochloric acid. Sample was heated again until the volume became 10 mL. Sample solution was diluted with deionized water until the volume eventually reached 100 mL. Blank samples were prepared by performing acid digestion without the sample. The investigation of seven heavy metal content (As, Cd, Pb, Co, Cr, Cu and Zn) was determined by Inductively Coupled Plasma-Mass Spectrometry, ICP-MS ELAN 9000, Perkin Elmer Sciex, USA with the suitable operating conditions shown in Table 2.

**Determination of total aerobic counts:** One gram of sample was dissolved in 9.0 mL sterile phosphate buffered saline solution. Serial dilutions were performed and 0.1 mL samples of the five final dilutions were spread on nutrient agar using a glass rod-shaped L. Nutrient agar was incubated at 37°C for 24-48 h. Total aerobic counts were determined by the following formula (Sharma, 2005):

Table 1: Details of ten herbal slimming products of different brands

Sample	Formulation	Daily dose (g X packet/ capsule/tablet)	Manufactory
A	Powder	7.00 X 1	Indonesia
B	Capsule	0.50 X 3	Indonesia
C	Capsule	0.35 X 2	Indonesia
D	Capsule	0.50 X 2	Indonesia
E	Tablet	0.50 X 3	Indonesia
F	Tablet	0.50 X 3	Malaysia
G	Capsule	0.50 X (4-6)	Malaysia
H	Capsule	0.50 X (4-6)	Malaysia
I	Tablet	0.75 X 2	Indonesia
J	Capsule	0.45 X 2	India

$$\text{Total aerobic counts (CFU g}^{-1}\text{)} = \frac{\text{DF} \times \text{CC}}{0.1 \text{ mL}}$$

where, DF is Dilution factor and CC is colony count.

**Identification of bacteria:** Gram staining was carried out from pure culture of bacteria, then, endospore staining was proceeded for Gram positive bacteria. Macroscopic observation was based on physical characteristics such as color, size, shape, edge, texture and surface colonies of bacteria on nutrient agar medium (Yap *et al.*, 1999; Bauman, 2007). Blood agar was used for inoculation of Gram positive bacteria and the hemolytic activity was observed (Bergey and Holt, 1994; Parry *et al.*, 1983). Meanwhile MacConkey and SS medium were used for Gram negative bacteria and lactose fermentation activity was observed. The formation of hydrogen sulfide with black spots production was observed on SS medium. Biochemical tests were performed to identify the bacteria, such as starch hydrolysis, catalase, citrate, MRVP, carbohydrate fermentation (glucose, sucrose, mannitol, lactose and arabinose), growth in 6.5% NaCl, motility, nitrate reduction, oxidase, TSI, indole and urease. The commercial kits of Microgen GN-ID A+B and API 20 E were used for Gram negative bacteria for confirmation.

Table 2: Operating conditions for ELAN 9000 ICP-mass spectrometer

RF power (W)	1000
Sampler diameter (mm)	1.1
Sample skimmer cone	Ni
Nebulizer (Mainhard)	Cross-flow
Peristaltic pump (mL min <sup>-1</sup> )	1
Argon flow rate plasma (L min <sup>-1</sup> )	15
Nebulizer flow (L min <sup>-1</sup> )	0.9
Spray chamber	Scott double pass

Table 3: Heavy metal contents (ppb, mean±SEM) of ten samples (1 g)

Sample	As	Cd	Cr	Cu	Zn
A	5.396±0.15	0.564±0.02	10.325±0.30	14.700±0.93	145.142±13.94
B	3.644±0.03	0.236±0.00	20.228±0.43	3.914±0.20	71.587±4.60
C	2.465±0.00	0.192±0.00	15.342±0.96	0.357±0.15	ND
D	1.815±0.01	0.067±0.00	32.020±0.74	ND	ND
E	2.836±0.02	0.065±0.01	6.327±0.15	ND	ND
F	4.340±0.09	0.072±0.00	5.967±0.03	ND	ND
G	4.168±0.05	0.016±0.01	8.630±0.07	ND	ND
H	2.974±0.02	ND	8.363±0.12	ND	ND
I	5.123±0.47	0.027±0.01	1.671±0.53	ND	ND
J	5.955±0.35	0.189±0.02	3.076±0.47	ND	ND

ND: Not detected, Pb and Co were not detected in all samples

Table 4: Daily consumption of heavy metal (ng day<sup>-1</sup>) based on manufacturers' recommended dose of ten samples

Sample	As	Cd	Cr	Cu	Zn
A	37.772	3.948	72.275	102.900	1015.994
B	5.466	0.354	30.342	5.871	107.381
C	1.726	0.134	10.739	0.250	ND
D	1.815	0.067	32.020	ND	ND
E	4.254	0.098	9.491	ND	ND
F	6.510	0.108	8.951	ND	ND
G	10.420	0.040	21.575	ND	ND
H	7.435	ND	20.908	ND	ND
I	7.685	0.041	2.507	ND	ND
J	5.360	0.170	2.768	ND	ND

ND: Not detected, Pb and Co were not detected in all samples

## RESULTS

**Investigation of heavy metal content:** Heavy metal content (ppb, Mean±Standard Error Mean (SEM)) of ten samples in 1 g was shown in Table 3. It was found that the content of lead (Pb) and cobalt (Co) were not detected in this study as these were below the detection limit. The content of arsenic (As) in all samples was in the range of 1.815-5.955 ppb. Sample J contained the highest concentrations of As (5.955±0.35 ppb) and followed by sample A (5.396±0.15 ppb) then sample I (5.123±0.47 ppb) whereas sample D had the lowest As content (1.815±0.01 ppb). The highest Cadmium (Cd) content was found in sample A (0.564±0.02 ppb) and the lowest in sample G (0.016±0.01 ppb) whereas sample H contained below the detection limit of Cd.

The chromium (Cr) content was between 1.671-32.020 ppb. The highest Cr content was found in sample D (32.020±0.74 ppb) and the lowest in sample I (1.671±0.53 ppb). For the copper (Cu), seven samples namely D, E, F, G, H, I and J had showed the absence of Cu. Sample A contained the highest content of Cu (14.700±0.93 ppb) followed by sample B (3.914±0.20 ppb) and sample C (0.357±0.15 ppb). Furthermore, range of zinc (Zn) contents in all samples was between 71.587-145.142 ppb. The highest Zn content was found in sample A (145.142±13.94 ppb) and followed by sample B (71.587±4.60 ppb). In addition, there were eight samples (C, D, E, F, G, H, I and J) had showed the absence of Zn.

Table 4 showed the daily consumption of heavy metal (ng day<sup>-1</sup>) based on manufacturers' recommended

Table 5: Total aerobic counts (CFU g<sup>-1</sup>) in ten samples

Sample	Total aerobic count (CFU g <sup>-1</sup> )
A	196×10 <sup>4</sup>
B	79×10 <sup>3</sup>
C	39×10 <sup>3</sup>
D	2×10
E	0
F	12×10
G	247×10 <sup>5</sup>
H	378×10 <sup>5</sup>
I	0
J	0

dose of ten samples. For heavy metals Pb and Co, no results for the daily consumption were obtained. Nevertheless, the range of As, Cd and Cr daily consumption was between 1.726-37.772, 0.040-3.948 and 2.507-72.275 ng day<sup>-1</sup>, respectively. It was found that sample A had the highest daily consumption of As and Cd. The lowest of As and Cd were found in sample C and G, respectively. There was no detection of Cd daily consumption was obtained from sample H. The lowest Cr daily consumption was found in sample I, while the highest was in sample A.

For Cu, the daily consumption of Cu was ranged between 0.250-102.900 ng day<sup>-1</sup>. Sample A showed the highest daily consumption, followed by sample B (5.871 ng day<sup>-1</sup>) then sample C (0.250 ng day<sup>-1</sup>). No result obtained for Cr daily consumption from samples D, E, F, G, H, I and J. In addition, the range of daily intake of Zn was 107.381-1015.994 ng day<sup>-1</sup> in which sample A contained the highest Zn daily consumption whereas sample B was showed the lowest value. No results obtained of daily Zn intake levels from samples C, D, E, F, G, H, I and J. Overall, sample A had the highest daily consumption of As, Cd, Cr, Cu and Zn.

**Total aerobic counts:** The total aerobic counts (CFU g<sup>-1</sup>) of ten samples were showed in Table 5. Sample H contained the highest value of total aerobic count (378 ×10<sup>5</sup> CFU g<sup>-1</sup>) then followed by sample G (247×10<sup>5</sup> CFU g<sup>-1</sup>) and sample A (196×10<sup>4</sup> CFU g<sup>-1</sup>). Three samples namely E, I and J had showed absence of bacteria. The order of total aerobic count obtained as followed: H>G>A>B>C>F>D>E, I and J.

**Identification of bacteria:** Nineteen bacteria spp. have been isolated in this study. Nine of them were Gram positive bacteria, eight of them were rod-shaped cell (A1, A3, B1, B2, C1, C2, C3 and C4) and one isolate with spiraling-shaped (A2). All nine gram positive isolates were endospore-forming bacteria with the presence of spores under microscopic observation. In this study, 10 Gram negative bacteria with rod-shaped cell namely A4, D1, D2, F1, F2, F3, F4, G1, G2 and H1 were able to isolate.

Table 6: Identification of Gram negative isolates using Microgen GN-ID A+B and API 20 E identification kits

Bacterial isolates	Code number	Bacteria (%)
A4	3305373	<i>Enterobacter sakazakii</i> (98.4%)
D1	520600401	<i>Burkholderia cepacia</i> (97.2%)
D2	000200401	<i>Myroides</i> spp./ <i>Chryseobacterium indologenes</i> (97.3%)
F1	520777705	<i>Raoultella planticola</i>
F2	220200427	<i>Pseudomonas aeruginosa</i> (97.9%)
F3	1027100	<i>Ewingella americana</i> (99.3%)
F4	1005100	<i>Ewingella americana</i> (98.7%)
G1	541170030	<i>Pseudomonas fluorescens</i> (95.36%)
G2	07441762	<i>Enterobacter agglomerans</i> (99.66%)
H1	000100430	<i>Brucella</i> spp.

Table 7: Identification of Gram positive isolates using biochemical tests

Bacterial isolates	Bacteria
A1	<i>Bacillus insolitus</i>
A2	<i>Bacillus anthracis</i>
A3	<i>Bacillus circulans</i>
B1	<i>Bacillus sphaericus</i>
B2	<i>Bacillus polymyxa</i>
C1	<i>Bacillus cereus</i>
C2	<i>Bacillus thuringiensis</i>
C3	<i>Bacillus badins</i>
C4	<i>Bacillus firmus</i>

All the gram negative isolates were identified through the application of commercial kits and the result were shown in Table 6. Meanwhile, nine isolates of gram positive were identified by using the biochemical test as shown in Table 7.

## DISCUSSION

Excessive doses or long term consumption of medicinal plants can lead to chronic accumulation of trace metals which may be harmful to the health of consumers. Chemical waste from factories can contribute heavy metal contamination to the herbs which are grown adjacent to those areas (WHO, 2007). The presence of excess heavy metal may interfere with metabolic functions (Singh *et al.*, 2011).

WHO (1999) had published a standard limitation of Cd and Pb which must less than 0.3 ppm (300 ppb) and 10 ppm (10 000 ppb), respectively. In this study, it was found out that heavy metals Cd and Pb level in all samples were below the standard limitation of WHO. Due to the long biological half-life, excessive accumulation of Cd can damage the kidneys and bones function, such as *Itai-itai* disease was found in Japan in year 1950 (Jarup *et al.*, 1998) and even high risk to kidney cancer (Kolonel, 1976).

Even though this study had found that as content in these ten samples was not exceed the standard limitation, but it can be toxic to the human which lead to abdominal pain and vomiting, while a long-term exposure can cause hyperkeratosis or skin pigmentation changes (Jarup, 2003) and even skin, lung, bladder and kidney cancer

(WHO, 2012). For Pb content, the limitations stated by the WHO, China and Thailand is 10,000 ppb, which is similar to Malaysia. However, none of the samples had detected Pb content in this study due to extreme low level. According to Jarup (2003), 50% of Pb was released into the air by the factor of petrol. Low Pb content is likely associated with the study of Shahid *et al.* (1987) who had stated that Government enforcement on reduction of Pb in petrol might efficiently to decrease the Pb releasing into the atmosphere. Proximal tubules damage in kidneys and nervous system disorders have occurred when a person is exposed to excessive Pb (WHO, 1992) and carcinogenic effects of Pb are stated in study of (Steenland and Boffetta, 2000). The low contents of As and Pb in this study can be attributed to the absorption of heavy metals in some herbal plants. This issue was accordance with the study carried out by Arpadjan *et al.* (2008), they were reported that the poor absorption rate of As and Pb from soil in some herbaceous plants and consumed As and Pb from herbal infusions are virtually not bioavailable.

Since there are no standard limitation prescribed in Malaysia for the content of chromium (Cr), copper (Cu), zinc (Zn) and cobalt (Co), these heavy metals were determined in value of daily consumption of heavy metals based on a recommended daily dose by the manufacturer. Daily consumption of heavy metals was compared with Tolerance Daily Intake (TDI), by Health Canada (2004).

In this study, the range of Cr daily consumption of all samples was within TDI value by Health Canada, 1 000 ng kg<sup>-1</sup>. Study by Miller-Ihli (1996) had shown Cr contamination could occur during the processing, packaging and transportation of herbs/herbal products. The use of stainless steel containing about 13-30% of Cr in the food processing equipment could contribute to contamination of Cr in the product. Study by Zayed and Terry (2003) had shown that chronic exposure to Cr might lead to liver and kidney impairment.

The range of Cu daily consumption of all samples was below TDI value, 141,000 ng kg<sup>-1</sup>. Nevertheless Georgopoulos *et al.* (2001) reported that human exposure to Cu from the water supply was a major pollutant sources. The presence of Cu in excess amount will interfere the gastrointestinal tract and can result in nausea, vomiting and diarrhea (Pizarro *et al.*, 1999). In addition, excessive Zn intake can be harmful and prevent the absorption of Cu and iron (Fe) in the body (Fosmire, 1990) as well as affect the nervous system (Alwakeel, 2008). However, the results of Zn daily consumption in this study was far below the standard limitations by WHO and was similar to the study of Alwakeel (2008) and Pizarro *et al.* (1999).

According to RIVM (2001), TDI value for Co was 0.0014 mg kg<sup>-1</sup> (1.4 mg kg<sup>-1</sup>) per body weight. However, the Co content was not detected in all samples because these were below the detection limit. When plants were cultivated nearby the mining and smelting facilities, they might contain high Co content. High consumption of Co can cause vomiting, nausea, visual disturbances, heart disease and deterioration of the thyroid (RIVM, 2001). Genotoxicity will occur by damaging DNA and interfering DNA repair mechanisms (De Boeck *et al.*, 2003). In this study, the total daily consumption of heavy metals was directly proportional to the daily dose taken by the product user. It showed that sample A (7 g per day) contained the highest daily consumption of several heavy metals compared to other samples.

Despite all the heavy metals tested in this study were within the standard limitation, it could not conclude that all samples in this study were safe and free of heavy metal contamination. Uptake of metals by plants can be influenced by several factors such as types of herbal plants, soil properties, climate and agricultural practices adopted by the producer of herbaceous plants (Tokalioglu, 2012). Alwakeel (2008) had indicated that the rate of heavy metal absorption by the body and the duration of use of herbal products may affect several adverse effects of metals on health. Besides, study by Tokalioglu (2012) found that heavy metal content was not distributed evenly in all parts of herbaceous plants. It might accumulate in certain parts of the plant, likewise the roots contained the highest levels of heavy metals and it was followed by plant tissue. This study was supported by Barthwal *et al.* (2008) who reported that levels of heavy metal contamination and permitted standard criteria should be evaluated based on the growth of herbaceous plants, plant species, the cultivation and production processes. Furthermore, the inconsistency between the groups of products could give different results (Ko, 1998). Study by Kunle *et al.* (2012) showed that the limitation of microbiological contamination was dependent to the final preparation of herbal products. Requirement of boiling water at the end process was an essential influence, since the heat-based decontamination system could heat the surface of a product and kill the pathogen very quickly at temperatures higher than 70°C (James *et al.*, 2007).

The presence of bacteria in this study was similar with the research conducted by McKee (1995) and WHO (2007), as the improper product handling and poor hygiene during the process of herbs planting, harvesting, storing and packaging could lead to this biological contamination. Mechanical disruption and solvent extraction during processing stage also affect the final

quality of herbal products (Mosihuzzaman and Choudhary, 2008). In addition, the adoption of Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP) specified in the study by Chan (2003) and Kosalec *et al.* (2009) may be ignored by the irresponsible manufacturer, in order to minimize the production costs and emphasize the company profits.

In addition, the presence of bacteria was closely related to high humidity levels in the raw source (Enayatifard *et al.*, 2010). If the harvesting process and collection of herbaceous plants be conducted during the rainy season, it might further promote the bacterial growth in herbaceous plants. Drying herbs on the ground under the sun can lead to microbiological contamination and this problem can be reduced by the application of closed drying system (Sagoo *et al.*, 2009). Studies by Sagoo *et al.* (2009) and Mishra *et al.* (2006), stated that the use of gamma radiation and dry heat sterilization could reduce microorganism's contamination in herbaceous plants. But, both methods might not used by manufacturers due to its high capital intensive.

Detection of *Enterobacter agglomerans* and the existence of bacteria susceptible to antibiotics such as *Bacillus* spp. and *Ewingella americana* from samples A, B, C, F and G was similar to the study by Kineman *et al.* (2002) and Brown and Jiang (2008). The presence of *Enterobacter sakazakii*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescent* in samples A, F and G were also supported by Baylis (2006) which found Enterobacteriaceae spp. and *Pseudomonads* spp. on the plants surface during harvesting process. *Enterobacter agglomerans* has existed widely in the environment and usually are not dangerous, but it has the ability to cause nosocomial infections (Geere, 1977).

The discovery of *Bacillus cereus* in this study was similar to the study by McKee (1995), Martins *et al.* (2001) and Alwakeel (2008). Biological contamination can occur when herbal cleaning process was not carried out properly. Eradicating dry powder contamination is difficult because *Bacillus* spp. are well-adapted itself to form spores in limited circumstances (Martins *et al.*, 2001). In addition, *Bacillus anthracis* is also commonly found in soil and can cause subcutaneous anthrax disease and inflammation when the spores enter the respiratory, gastrointestinal or skin (Schneider *et al.*, 2004; Koehler, 2009).

In this study, although total aerobic count of sample C and F did not exceed the standard limitations by WHO, the presence of *Bacillus cereus* and *Pseudomonas aeruginosa* might be the potential cause of food poisoning. These results are similar to past research study

which observed by Enayatifard *et al.* (2010), that the total aerobic count is not directly proportional to the presence of pathogenic bacteria. However, this was associated with low hygiene levels of product handling.

## CONCLUSION

The bacteria content had exceed the standard in certain samples with the presence of the potential food poisoning causing bacteria however, heavy metals content in all the samples are under the permissible level. Good handling practice has required to ensure all the products in the market was safe for the users and consumers.

## ACKNOWLEDGMENT

This was supported by The UKM Research University Grant under project code OUP-2012-100.

## REFERENCES

- Alwakeel, S.S., 2008. Microbial and heavy metals contamination of herbal medicines. Res. J. Microbiol., 3: 683-691.
- Arpadjan, S., G. Celik, S. Taskesen and S. Gucer, 2008. Arsenic cadmium and lead in medicinal herbs and their fractionation. J. Food Chem. Toxicol., 46: 2871-2875.
- Athar, M. and S.B. Vohora, 1995. Heavy Metals and Environment. 1st Edn., New Age International Publishers, New Delhi, ISBN: 81-224-0769-2.
- Banerjee, M. and P.K. Sarkar, 2003. Microbiological quality of some retail spices in India. Food Res. Int., 36: 469-474.
- Barthwal, J., S. Nair and P. Kakkar, 2008. Heavy metal accumulation in medicinal plants collected from environmentally different sites. Biomed. Environ. Sci., 21: 319-324.
- Bauman, R.W., 2007. Microbiology with Disease by Taxonomy. 2nd Edn., Pearson Benjamin Cummings, San Francisco.
- Baylis, C.L., 2006. Enterobacteriaceae: Food Spoilage Microorganisms. Wood Head Publishing, United Kingdom.
- Bergey, D.H. and J.G. Holt, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams Wilkins, Baltimore, USA., ISBN: 9780683006032, Pages: 787.
- Brown, J.C. and X. Jiang, 2008. Prevalence of antibiotic-resistant bacterial on herbal products. J. Food Protect., 71: 1486-1490.

- Chan, K., 2003. Some aspects of toxic contaminants in herbal medicines. *Chemosphere*, 52: 1361-1371.
- De Boeck, M., M. Kirsch-Volders and D. Lison, 2003. Cobalt and antimony: Genotoxicity and carcinogenicity. *Mutation Res.*, 533: 135-152.
- De Smet, P.A.G.M., 2004. Health risks of herbal remedies: An update. *Clin. Pharmacol. Therapeut.*, 76: 1-17.
- Enayatifard, R., H. Asgarirad and B. Kazemi-Sani, 2010. Microbial quality of some herbal solid dosage forms. *Afr. J. Biotechnol.*, 9: 1701-1705.
- Ernst, E. and A. White, 2000. The BBC survey of complementary medicine use in the UK. *Complementary Therapies Med.*, 8: 32-36.
- Ernst, E., 2002. Toxic heavy metals and undeclared drugs in asian herbal medicine. *Trends Pharmacol. Sci.*, 23: 136-139.
- Fosmire, G.J., 1990. Zinc toxicity. *Am. J. Clin. Nutr.*, 51: 225-227.
- Geere, I.W., 1977. Enterobacter agglomerans: The clinically important plant pathogen. *Can. Med. Assoc. J.*, 116: 517-519.
- Geok, L.K., 2012. Food availability and the rising obesity prevalence in Malaysia. *Int. J. Sci. Med. Educ.*, 6: S61-S68.
- Georgopoulos, P.G., A. Roy, M.J. Yonone-Lioy, R.E. Opiekun and P.J. Lioy, 2001. Environmental copper: Its dynamics and human exposure issues. *J. Toxicol. Environ. Health: Part B Crit. Rev.*, 4: 341-394.
- Health Canada, 2004. Federal contaminated site risk assessment in Canada. Part II: Health Canada toxicological reference values (TRVs). Environmental Health Assessment Services Safe Environments Programme, Ottawa.
- ICMSF, 2005. Spices, Herbs and Vegetable Seasonings. In: *Micro-Organisms in Foods 6: Microbial Ecology of Food Commodities*, Roberts, T.A. (Ed.). 2nd Edn., Springer, London.
- Institute of Public Health, 2008. Nutritional status (the third national health and morbidity survey 2006). Ministry of Health Malaysia, Malaysia.
- Izydorczyk, B., 2011. A psychological diagnosis of the structure of the body self in a group of selected young Polish females without eating or other mental disorders. *Arch. Psychiatry Psychotherapy*, 2: 21-30.
- James, C., S.J. James, N. Hanay, G. Purnell and C. Barbedo-Pinto *et al.*, 2007. Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of carcass surfaces. *Int. J. Food Microbiol.*, 114: 195-203.
- Jantan, I., 2006. The scientific values of Malaysian herbal products. *J. Sains Kesihatan Malaysia*, 4: 59-70.
- Jarup, L., M. Berglund, C.G. Elinder, G. Nordberg and M. Vahter, 1998. Health effects of cadmium exposure—a review of the literature and a risk estimate. *Scand J. Work Environ. Health*, 24: 1-51.
- Jarup, L., 2003. Hazards of heavy metal contamination. *Br. Med. Bull.*, 68: 167-182.
- Kineman, B., M.L. Nahikian-Nelms and C. Frazier, 2002. A pilot investigation of the microbial contamination of herbal supplements: A potential risk for immunocompromised populations. *HIV Nutr. Update*, 7: 1-9.
- Ko, R.J., 1998. Adulterants in Asian patent medicines. *New England J. Med.*, 339: 847-847.
- Koehler, T.M., 2009. *Bacillus anthracis* physiology and genetics. *Molecular Aspects Med.*, 30: 386-396.
- Kolonel, L.N., 1976. Association of cadmium with renal cancer. *Cancer*, 37: 1782-1287.
- Kosalec, I., J. Cvek and S. Tomic, 2009. Contaminants of medical herbs and herbal products. *Arch. Ind. Hygiene Toksikol.*, 60: 485-501.
- Kunle, O.F., H.O. Egharevba and P.O. Ahmadu, 2012. Standardization of herbal medicines—A review. *Int. J. Biodivers. Conserv.*, 4: 101-112.
- Martins, H.M., M.L. Martins, M.I. Dias and F. Bernardo, 2001. Evaluation of microbiological quality of medicinal plants used in natural infusions. *Int. J. Food Microbiol.*, 68: 149-153.
- McDaniel, W.D., 1991. Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements in Biological Tissues: Test Method 200.3. U.S. Environmental Protection Agency, USA.
- McIntyre, T., 2003. Phytoremediation of heavy metals from soils. *Adv. Biochem. Eng. Biotechnol.*, 78: 97-123.
- McKee, L.H., 1995. Microbial contamination of spices and herbs: A review. *LWT-Food Sci. Technol.*, 28: 1-11.
- Miller-Ihli, N.J., 1996. Graphite furnace atomic absorption spectrometry for the determination of the chromium content of selected US foods. *J. Food Composition Anal.*, 9: 290-300.
- Mishra, B.B., S. Gautam and A. Sharma, 2006. Microbial decontamination of tea (*Camellia sinensis*) by gamma radiation. *J. Food Sci.*, 71: M151-M156.
- Mosihuzzaman, M. and M.I. Choudhary, 2008. Protocols on safety, efficacy, standardization and documentation of herbal medicine. *Pure Appl. Chem.*, 80: 2195-2230.
- Parry, J.M., P.C.B. Turnbull and J.R. Gibson, 1983. A Colour Atlas of *Bacillus* species. Wolfe Medical Publications Ltd., London, UK., ISBN-13: 9780723407775, pp: 272.



- Pizarro, F., M. Olivares, V. Gidi and M. Araya, 1999. The gastrointestinal tract and acute effects of copper in drinking water and beverages. *Rev. Environ. Health*, 4: 231-238.
- RIVM, 2001. Re-evaluation of human-toxicological maximum permissible risk levels. National Institute of Public Health and the Environment. Netherlands, Bilthoven.
- Riji, H.M., 2006. Beauty and health? A personal view. *Malaysian Family Phys.*, 1: 42-44.
- Sagoo, S.K., C.L. Little, M. Greenwood, V. Mithani and K.A. Grant *et al.*, 2009. Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. *Food Microbiol.*, 26: 39-43.
- Schneider, K.R., M.E. Parish, R.M. Goodrich and T. Cookingham, 2004. Preventing Foodborne Illness: *Bacillus cereus* and *Bacillus anthracis*. Florida Cooperative Extension Services, EDIS FSHN036. University of Florida, Amerika Syarikat.
- Shahid, S.M., E.S. Tee and Y.H. Chong, 1987. Lead content of some Malaysian foodstuffs. *Asean Food J.*, 3: 25-29.
- Sharma, P.D., 2005. *Environmental Microbiology*. Alpha Science International Ltd., Harrow, UK.
- Singh, R., N. Gautam, A. Mishra and R. Gupta, 2011. Heavy metals and living systems: An overview. *Indian J. Pharmacol.*, 43: 246-253.
- Steenland, K. and P. Boffetta, 2000. Lead and cancer in humans: Where are we now? *Am. J. Ind. Med.*, 38: 295-299.
- Tan, A.K.G., R.A. Dunn, A.S. Mohamed Ismail and I.F. Mustapha, 2011. Sociodemographic and health-lifestyle determinants of obesity risks in Malaysia. *Asia-Pacific J. Public Health*, 23: 192-202.
- Tokalioglu, S., 2012. Determination of trace elements in commonly consumed medicinal herbs by ICP-MS and multivariate analysis. *Food Chem.*, 134: 2504-2508.
- Uluozlu, O.D., M. Tuzen, D. Mendil and M. Soyak, 2009. Assessment of trace element contents of chicken products from Turkey. *J. Hazard. Mater.*, 163: 982-987.
- WHO, 1992. Cadmium, *Environmental Health Criteria*, Vol. 134. International Progress in Chemical Safety. WHO, Geneva.
- WHO, 1999. WHO Monographs on Selected Medicinal Plants. Vol. 1, World Health Organization, Geneva.
- WHO, 2004. WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. World Health Organization, Geneva, ISBN: 9789241592215, Pages: 18.
- WHO, 2007. WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues. World Health Organization, Geneva, ISBN: 9241594446, Pages: 105.
- WHO, 2012. Arsenic. Fact Sheet No. 372, December 2012. <http://www.who.int/mediacentre/factsheets/fs372/en/>
- Witkowska, A.M., D.K. Hickey, M. Alonso-Gomez and M.G. Wilkinson, 2011. The microbiological quality of commercial herb and spice preparations used in the formulation of a chicken supreme ready meal and microbial survival following a simulated industrial heating process. *Food Control*, 22: 616-625.
- Yap, K.L., A.H.A. Aziz and M.S.M. Yasin, 1999. *Mikrobiologi makmal*. Penerbit Universiti Kebangsaan Malaysia, Bangi.
- Zayed, A.M. and N. Terry, 2003. Chromium in the environment: Factors affecting biological remediation. *Plant Soil*, 249: 139-156.