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Screening of adulterants in unregistered herbal products in Malaysia

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ABSTRACT

Global increased demand for herbal medicine in recent years has resulted in the raise of concerns over its quality and safety. Tighter regulations and surveillance in many Asian countries have also been imposed in order to protect public's health interest. The aim of the present work was to screen for possible adulterants including heavy metals, prescription drugs, poisons and steroids contamination in unregistered herbal products claimed to possess desired medicinal values. These products were obtained via enforcement raids from several bazaars in Malaysia and analyzed by means of analytical techniques: GC-MS, HPLC-PDA and AAS. The levels of Hg (≤ 0.30 mg/kg), Pb (≤ 3.41 mg/kg) and As (≤ 0.50 mg/kg) found were below the permissible limits (i.e., 0.5, 10 and 5 mg/kg, respectively), and none of the known dangerous drugs, poisons or steroids was detected. However, all samples were found to contain an undeclared synthetic PDE-5 inhibitor, sildenafil or tadalafil, in medium to extremely high doses while two samples also contained paracetamol. As such, the public is cautioned and should not purchase unregistered herbal products because unsupervised consumption of any synthetic PDE-5 inhibitor in extremely high doses may have serious health implications.

Keywords: Herbal medicine, heavy metals, drugs, poisons, synthetic PDE-5 inhibitors

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INTRODUCTION

Herbal preparations have long been used for the prevention and treatment of diseases since the beginning of human civilization. To date, they remain as the primary source of healthcare for approximately 80% of the world's population [1] and the demand for these products is increasing in developed countries [2]. Among the reasons for the popular demand for herbs include user's disappointment towards orthodox medicine in providing effective and holistic treatment on certain ailments, perceived effectiveness of complementary and alternative medicine, soothing properties of herbs, cultural and personal beliefs, as well as user's regard on the inherent safety of herbal medicine [3-5]. While many users are claiming the beneficial effect of herbal medicines, reports on deleterious effects associated with the consumption of herbal products are also appearing from time to time. A large proportion of these adverse effects were attributed to the poor quality of the finished products, contamination with heavy metals, pesticides, microorganism and mycotoxins, adulteration with drugs, as well as misidentification of herbs or substitution of herbs with those of superior quality [6, 7].

In Malaysia, owing to the strong influence from a confluence of traditional medicinal systems, the use of raw herbs and/or herbal preparations is often a part and parcel of daily life. Herbal medicines in the form of dietary supplements, although do not require to undergo rigorous tests to provide proof on their efficacy, safety and quality, these products are required to be registered with the Drug Control Authority (DCA) of Malaysia where adulteration of drugs and contamination with heavy metals and microorganisms are being monitored [8]. However, many unregistered herbal products claimed to possess good medicinal values, either tacitly or explicitly, are still loosely

available in the market streets. These products are either manufactured locally or imported from neighboring Asian countries. As recent reports have implicated that some Chinese and Indian preparations contained alarming quantity of toxic heavy metals and undeclared drug substances [9], the aim of the present study is to screen for potential health hazards in unregistered herbal products obtained from the market streets in Malaysia. The analyses include the detection of poisons, drugs, corticosteroids and sex hormones, as well as quantification of synthetic PDE-5 inhibitors and heavy metals.

MATERIALS AND METHODS

2.1. Samples

51 samples consisting tablets, capsules and herbal powders of different packaging, color and composition were seized by the law enforcement authorities from several bazaars in the northern part of Peninsular Malaysia and submitted to the Department of Chemistry Malaysia, Penang. The main intention of the present study was to focus on the analysis of the herbal powder samples, of which there were five. These products were apparently manufactured in Indonesia and packed in plastic sachets with total weights ranging from 7 to 10 g. Their compositions were dubiously declared to consist of only natural ingredients such as ginseng and root extracts, while their trade name seemed to imply that the products may provide 'extra power'.

2.2. Chemicals

Sildenafil citrate, tadalafil and vardenafil hydrochloride standards were obtained from the National Pharmaceutical Bureau of Malaysia while diazepam was obtained from the Research and Quality Assurance Division, Department of Chemistry, Malaysia. The corticosteroids and sex hormones standards: prednisolone, hydrocortisone, betamethasone, dexamethasone, triamcinolone, ethinyl estradiol, beclomethasone, fluocinolone and levonorgestrel were also obtained from the National Pharmaceutical Bureau of Malaysia. The acetonitrile and methanol used were of HPLC grade purchased from Fisher Scientific and Merck, respectively. Analytical grade ethanol was purchased from Merck while triethylammonium phosphate buffer solution 1M was purchased from Fluka.

2.3. Screening for common poisons/drugs by GC-MS

50 mg sample was taken from a homogenous mixture of several sachets of each herbal product. The sample was then extracted with 1 mL methanol with the assistance of ultrasound and then centrifuged at 2000 r.p.m. for 10 min. The resulting supernatant was transferred into a 2 mL glass vial for analysis by means of hyphenated gas chromatography and mass spectrometry system (GC-MS). The GC-MS system consisted of an Agilent 6890 Gas Chromatograph and Agilent 5973 Mass Selective Detector (Agilent Technologies, Germany). The capillary column was HP-5 MS, 30 m × 0.25 mm with 0.25 µm film thickness. The injector and auxiliary temperatures were set at 260 °C and 280 °C, respectively. The flow rate of the helium gas was 1.5 mL/min. Initial oven temperature was 80 °C and this was gradually increased at a rate of 20 °C/min until a maximum temperature of 320 °C, where it was held constant for 5 min. A scan acquisition mode from 50 to 550 a.m.u. was applied in this analysis. Spectra match was then performed using the NIST05 search library to screen for known poisons and prescription drugs which might have been added deliberately to these products.

2.4. Screening for corticosteroids/sex hormones by HPLC-PDA

100 mg sample was taken from a homogenous mixture of several sachets of each herbal product. The sample was extracted with 50% methanol in aqueous with the aid of ultrasonication and warmed on a water bath at 45°C for 20 min. Following that, the sample extract was allowed to cool to room temperature and an aliquot of 1 mL of the extract was filtered through a 0.45 µm syringe filter into a 2 mL glass vial. A Waters 2695 Separation Module coupled to a 2996 Photodiode Array Detector (PDA) was used for the analysis (Waters Corp., USA). A Kinetex XB-C18 HPLC column, 50 × 4.60 mm, 2.6 µm (Phenomenax, USA) was used as the stationary phase. The temperature of the column was maintained at 40 °C and the sample injection volume was 15 µl. The mobile phase consisted of acetonitrile (A) and ultrapure water (B) flowing at 1.2 mL/min in a step gradient mode. The composition of A was 25% at 0 min, linearly increased to 30% at 5 min, followed by further increment to 50% at 12 min where it was held constant until 19 min, and subsequently returned to 25% at 21 min. Identification of the steroids and hormones was done by comparing the retention time and UV spectra of the analytes in the range of 210 - 400 nm against a mixed reference standard solution containing 25 µg/mL of prednisolone, hydrocortisone, dexamethasone, triamcinolone, fluocinolone, cortisone, ethinyl estradiol, levonorgestrel, betamethasone and beclomethasone.

2.5. Quantification of PDE-5 inhibitors by HPLC-PDA

50.00 mg of each product sample was weighed into a centrifuge tube. The sample was then extracted with 1 mL ethanol containing diazepam as the internal standard at the concentration of 250 µg/mL. The extraction procedure was carried out with the aid of ultrasonication for 15 min at room temperature (ca. 25 °C). Following that, the extract was centrifuged at 2000 r.p.m. for 10 min. 400 µL aliquot of the supernatant was then transferred into a 10

mL volumetric flask and the solution was made up to the volume with 10% ethanol. The sample solution was filtered using a 0.45 µm syringe filter prior to injecting into the HPLC system. Quantification of sildenafil, tadalafil and vardenafil was performed based on the calibration curves of these reference standards in the concentration range of 10 -140 µg/mL.

The HPLC instrumentation consisted of a Waters 2695 Separations Module which was coupled to a Waters 2996 Photodiode Array Detector (Waters Corp., USA). Chromatographic separation was achieved on a Kinetex XB-C18 column, 50 × 4.60 mm, 2.6 µm (Phenomenex, USA) maintained at 40 ^{o}C, where the mobile phase consisted of acetonitrile (A) and 0.01M TEAP (B) flowing at 1.3 mL/min in a step gradient mode. The initial mobile phase composition of A was 30% for 5 min, followed by a linear increment to 75% from 5-10 min and then returning to 30% from 10-12 min where it was then held isocratic for a period of 2 min. The sample injection volume was 15 µL. Detection was done in the range of 200-400 nm and UV signals were extracted at 230 nm. Method validation had been carried out and found that the present method was suitable for reliable quantification of sildenafil, tadalafil and vardenafil in the range of 10 – 140 µg/ml (data presented elsewhere).

2.6. Quantification of heavy metals by AAS

Quantification of mercury, lead and arsenic in the herbal products was performed using Atomic Absorption Spectroscopy (AAS), in accordance to the procedure provided by the American Public Health Association (APHA) which was validated in house. In brief, 0.5 g of each of the five herbal product samples was weighed individually into a beaker followed by addition of 10 mL ultrapure water. An acid digestion method was then performed by adding 5 mL of concentrated HNO_3, 2.5 mL of H_2SO_4 and 1.0 mL of HCl to the samples and left for 2 days on a water bath set at 80 °C. Following that, the samples were filtered using a Whatman 4 qualitative filter paper into 50 mL volumetric flasks and made up to volume with ultrapure water prior to analysis using a PinAAcle 900F flame atomic absorption spectrometer (Perkin Elmer, USA). Cableless Lumina hollow cathode lamps of mercury, lead and arsenic were used as the light source, while a deuterium lamp was used for background correction. The flame conditions were optimised using oxidant flow of 10.0 L/min and acetylene flow of 2.5 L/min.

RESULTS

3.1. Poison and drugs screening

The herbal products were analyzed for the presence of prescription drugs such as anti-convulsants, anti-depressants, anti-histamines, analgesics, non-steroidal anti-inflammatory drugs, and anaesthetics. Out of the five herbal products, two were found to be adulterated with paracetamol. A representative GC-MS data of a herbal product sample that contained paracetamol is shown in Fig. 1. Other prescription drugs screened were not detected.

3.2. Corticosteroids screening

A simple and reliable HPLC-PDA method for simultaneous detection of 10 commonly available corticosteroids had been used for this analysis. The herbal products were screened against a reference standard injection that consisted of a mixture of prednisolone, hydrocortisone, dexamethasone, triamcinolone, fluocinolone, cortisone, ethinyl estradiol, levonorgestrel, betamethasone and beclomethasone. As shown in Fig. 2, none of the herbal samples were found to contain the corticosteroids.

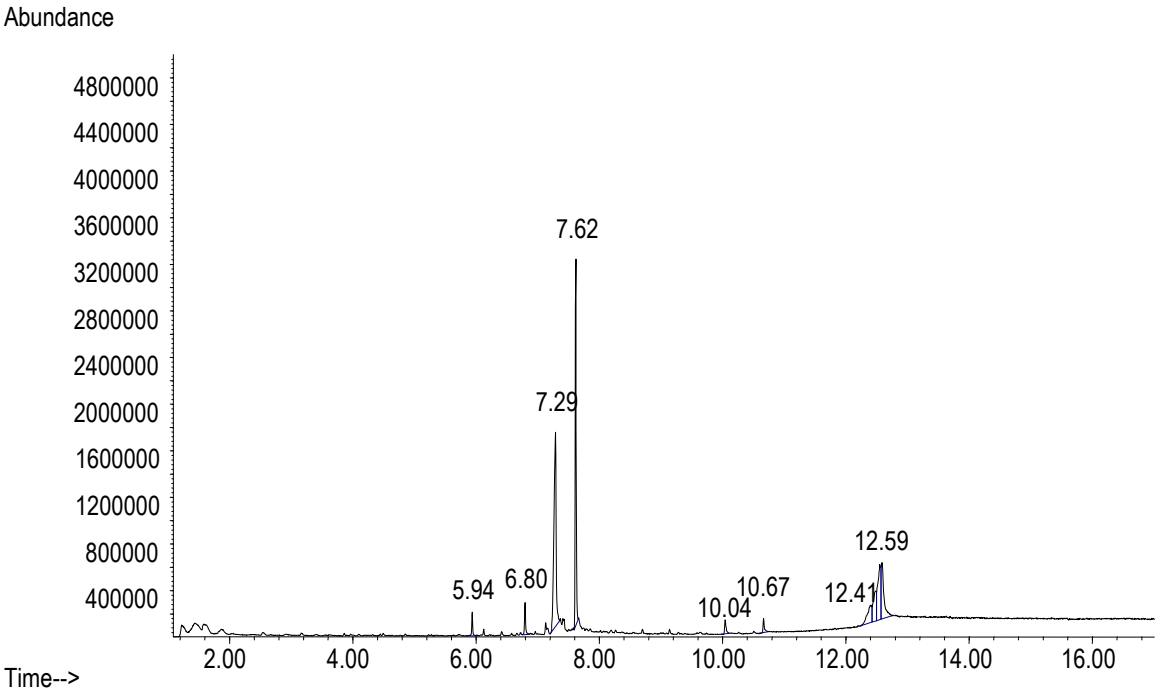
3.3. Quantification of PDE-5 inhibitors

The detection and quantification of sildenafil and tadalafil in the herbal products was performed on a validated HPLC-PDA system. Sildenafil was found in four while taladafil was found in one of the herbal products. The concentration of the detected drugs range from 5.40 – 27.56 mg/g, and when converted to the amount per dose based on the serving size suggested, the amount of drug per intake ranged from 37.78 – 275.60 mg (Table 1).

3.4. Quantification of heavy metals

Quantification of lead, arsenic and mercury performed on the AAS system revealed that all five herbal products contained low levels of these elements. The amount of lead present was found to be in the range of 2 – 4 ppm, while the amount of arsenic and mercury was ≤ 0.5 ppm (Table 2).

(A)



(B)

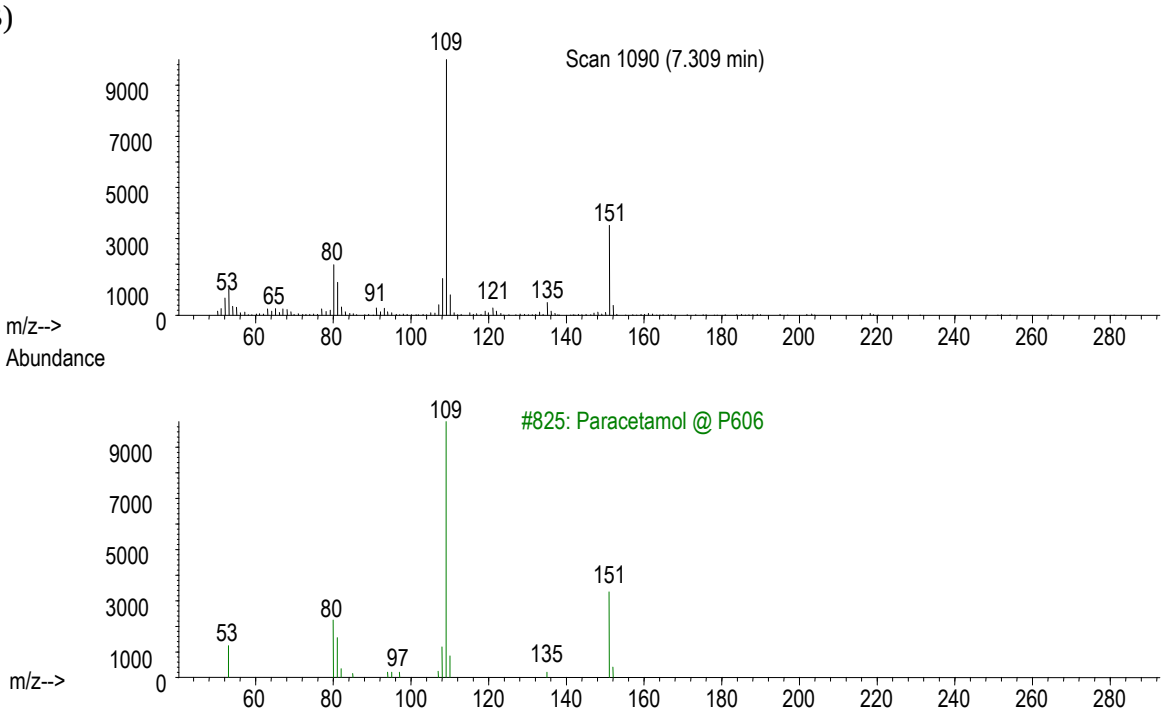


Figure 1: GC-MS data of a herbal product sample that contained paracetamol: (A) total ionic chromatogram; (B) mass spectra of the sample (top) against spectra of paracetamol from NIST library (bottom).

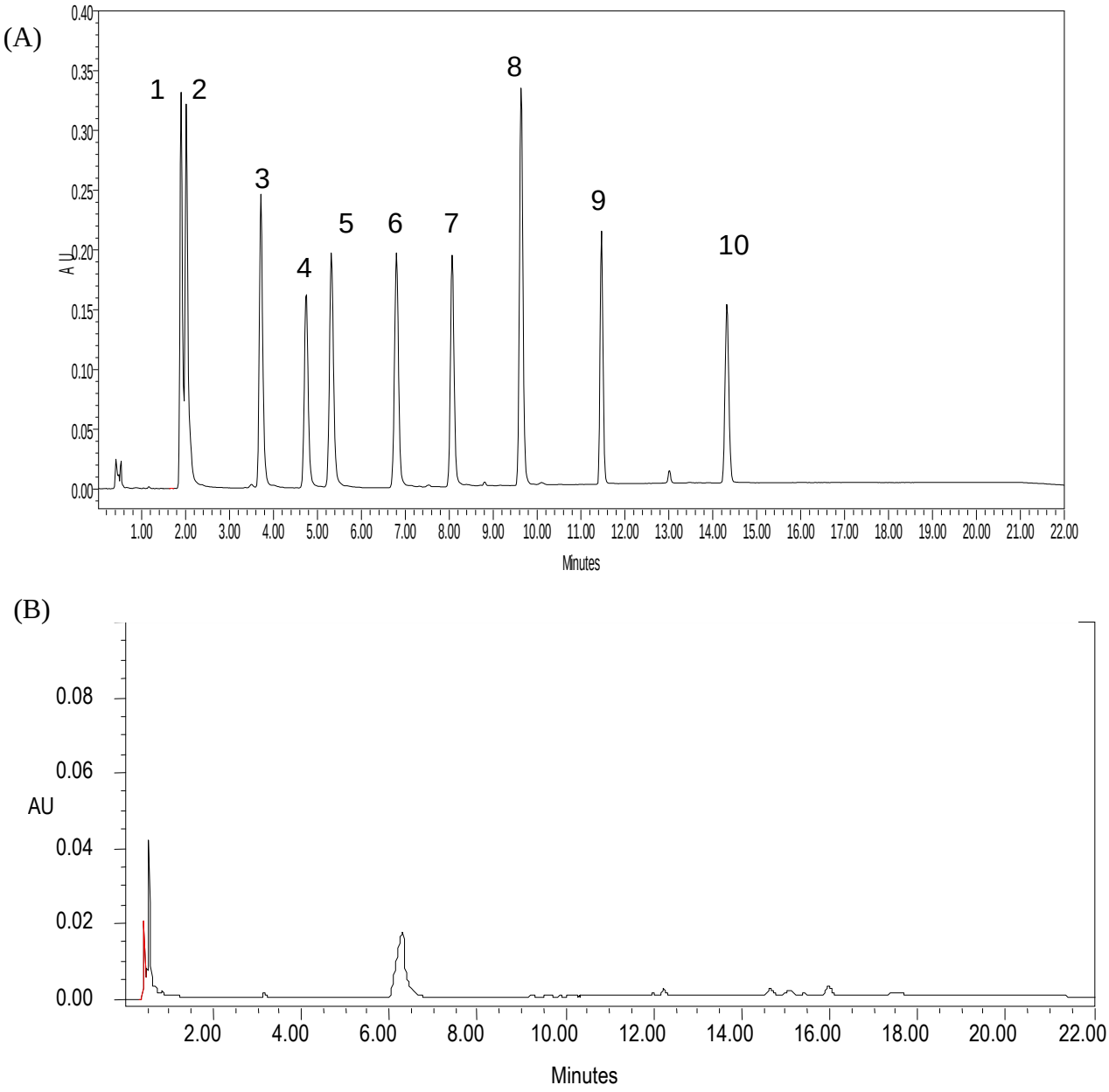


Fig. 2: HPLC chromatograms: (A) mix standards comprised of (1) prednisolone, (2) hydrocortisone, (3) dexamethasone, (4) triamcinolone, (5) fluocinolone, (6) cortisone, (7) ethinyl estradiol, (8) levonorgestrel, (9) betamethasone and (10) beclomethasone at 25 µg/ml; (B) a herbal product sample.

Table 1: Synthetic PDE-5 inhibitors detected and quantified in the herbal product samples

Sample No.	Sample appearance	Serving size, g	Analyte detected*	Analyte concentration, mg/g	Quantity per dose, mg
1		7.0	S	5.40	37.78
2		7.0	S	6.95	48.65
3		7.0	T	12.21	85.49
4		7.0	S	17.86	125.02
5		10.0	S	27.56	275.60

** S = sildenafil; T = tadalafil*

Table 2: Heavy metals content in the herbal product samples

Sample no.	Concentration of heavy metals (mg/kg)		
	Hg	Pb	As
1	0.30	2.77	0.48
2	0.10	3.41	0.50
3	0.16	2.16	0.40
4	0.18	2.57	0.40
5	0.12	2.70	0.46

DISCUSSION

Herbal products used in traditional medicine generally contained low concentrations of complex chemical constituents which are difficult to identify, characterize and standardize and therefore they cannot be accessed in a manner similar to orthodox medicine [2]. As such, the safety and quality of these herbal products are regulated according to the risk-based approach. Among the quality issues associated with external factors that are often accessed by the regulatories include contamination with heavy metals, pesticides, microorganisms, mycotoxin and other non-herbal based foreign matters. Another issue commonly encountered is adulteration with orthodox drugs [10]. Such action is done deliberately in order to produce therapeutic effect or to potentiate the effects of herbal medicine so as to attract customers [11]. According to the report from the Ministry of Health Malaysia, approximately 37% of patients diagnose with renal problem in the country were attributed to long term consumption of herbal products, of which many contained steroids, drugs, poisons and heavy metals [12]. The chances of unregistered herbal products to be adulterated with steroids itself were found to be as high as 95% [13]. This is an alarming rate and thus calls for more stringent surveillance on unregistered herbal products.

The present work was carried out to evaluate five unregistered herbal products in the form of powder obtained from an enforcement raid. These products were screened for possible drug adulterants such as anti-convulsants, anti-depressants, anti-histamines, analgesics, non-steroidal anti-inflammatory drugs, anaesthetics and the like. Based on our findings, no dangerous drug or poison was found. Two of the herbal products were found to contain paracetamol, which is a widely used over-the-counter analgesic. All five herbal products were detected with synthetic PDE-5 inhibitors – four with sildenafil and one with tadalafil, but no nitrates were present which may otherwise interact with these drugs, leading to significant hypotension [14]. We proceeded to quantify the amount of these synthetic PDE-5 inhibitors and found that their quantity vary from medium to high concentrations (Table 1). Based on the recommended dose regimen for sildenafil at 50 mg per day [15] and tadalafil at 20 mg taken on an on-demand basis or 3 times/week basis [16], samples no. 3, 4 and 5 are considered as having extremely high doses of synthetic PDE-5 inhibitors. According to the guidelines listed by the Drug Control Authority of Malaysia, an anti-impotence drug can only be prescribed by a medical doctor registered with the Malaysian Medical Council after clinical evaluation to confirm the diagnosis of erectile dysfunction and the appropriateness of drug therapy [17]. Unsupervised consumption of PDE-5 inhibitors may result in adverse reactions such as headache, flushing, dyspepsia, dizziness and vision disturbances [18], while studies have shown that long term exposure to overdoses of sildenafil leads to significant alteration of the morphology and histology of testes, which may eventually lead to complete arrest of spermatogenesis in animals [19].

The permissible limits of mercury, lead and arsenic in herbal preparations as stated in the Poisons Act 1952 are 0.5, 10 and 5 mg/kg, respectively. The present results have shown that all five herbal products contained heavy metals below the regulated limit. Since the contamination of any given herbal product with heavy metals is often associated with poor environmental control and pollution during production, unless it is deliberately added [7], the results of this study suggest that there is some form of quality control throughout the process of manufacturing.

CONCLUSION

In conclusion, the five unregistered herbal products that seemed to be originated from Indonesia do not contain dangerous drugs, poison or exceeding levels of heavy metals. However, two of the herbal products contained paracetamol, while all contained medium to extremely high levels of synthetic PDE-5 inhibitors. Based on the analytical results, coupled with the implication of the product names, it is clear from this study that the manufacturers and/or the agent of these products have clandestine intention to deceive and satisfy customers who are seeking for better sexual performance. However, these vendors have little understanding on the use of optimal drug dosage to achieve cost- effective results, thus most of these products were overdosed – putting the health of consumers at stake. The public should be cautioned on the danger of purchasing aphrodisiac supplements from the market streets and that medical advice should be sought prior to consuming any unregistered herbal products.

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