Evaluation of Heavy Metals and Microbiological Contamination of Selected Herbals from Palestine

1 Introduction

Large sections of the population in developing countries still rely on herbal medicines for their primary care. Traditional medicine is widely used for the prevention and treatment of many diseases, and are also used to boost energy and improve the immune system [1, 2].

In many countries including Africa, India and China their populations rely on traditional medicine to meet their health care needs [3]. Nowadays herbal medicines are widely present in herbal traditional shops, supermarkets as well as community pharmacies. The herbal medicine is present in different forms such as herbal bags, creams, powder etc. In Palestine, herbal medicine is considered an integral part of Palestinian culture and plays an important role in current public healthcare. The Palestinian hills and mountains are covered with more than 2600 plant species of which about 700 of them are of known medical uses [4].

The limits of toxic metals in the form of impurities depend on the nature of the sample and the contaminants or residues. The national limits for toxic metals and microbial contamination in various types of herbal products are different for each country and depend on the herb type and whether it is raw material or a finished product [5].

The WHO has put specification for microbial limits including aerobic bacteria, yeast and Escherichia coli. The limit varies according to the intended use of the herbs. In general the allowable limits are more when the herbs are intended for external use or require boiling before administration [6].

The use of medicinal herbs in therapeutic remedies is a common practice among Palestinians. Many of the herbs used are mainly cultivated and produced in Palestine; these herbs include: Sicilian Sumac, Oleander, Palestinian Arum, Hawthorn and Cardamom [7]. However, many of the herbs used are imported from African countries such as Egypt or from South Asia such India. Herbal medicine in Palestine is widely used in the treatment of GI Disturbances, cardiovascular, sedation and weight control [8-10].
Some of the widely used herbs in traditional medicine in Palestine include: *Matricaria chamomilla* L. (Chamomile) is a plant with white flowers, it is commonly used for the relief of inflammation, ulcers, muscle spasm, gastrointestinal disorders, hemorrhoids, menstrual disorders and insomnia [11]. *Pimpinella anisum* L. (Anise) has small green to yellow seeds, it is commonly used as muscle relaxant, analgesic and has beneficial effects for dysmenorrhea and menopausal hot flushes in women. In diabetic patients, aniseeds have a hypoglycemic and hypolipidemic effect [12]. *Zingiber officinale* Rosco (Ginger) is the horizontal stem from which the roots grow and the main portion of it consumed. Ginger is commonly used for arthritis, rheumatism, muscular aches, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia and have anti hyperglycemic effect [13]. *Crataegus azarolus* L. (Hawthorn) is a low, dense, spiny tree with orange fruit the fruits, flowers and leaves are the parts used. It is used for control of hyperglycemia with its associated complications[14]. *Hibiscus sabdariffa* L. (Roselle) is fleshy and develops bright red flowers as the fruit matures. It has antioxidant, hypotensive, and anti-atherosclerotic effects [15].

Herbs in Palestine are usually sold in the traditional medicine shops and some of them are sold in pharmacies. The Ministry of Health (MoH) is the authority that monitors the medicinal plants market in Palestine. The rules to control the herbal medicines are newly established. The general directorate of pharmacy in MoH is the department that takes the responsibility of importing, exporting and producing the medicinal herbs in the Palestinian market. A set of rules has been recently issued which include that any store selling or importing herbs must be licensed by the ministry of health. The store must employ a responsible pharmacist, chemist, medical biologist, food engineering or chemical engineer. The ministry of health has the right to withdraw any herb proved to have a dangerous side effect based on WHO recommendations. However, the MoH have not yet established rules and limitations for bacterial and elemental contamination of herbs. The wide used of herbal medicine in Palestine makes it necessary to perform regular investigation and to make a quality control check for the microbial and heavy metal content of herbs sold in the Palestinian markets [16].

Many studies were conducted all over the world to evaluate herbal heavy metal and microbial contamination. Studies were conducted in countries like Ghana, Japan, Ayurveda, Nigeria, Brazil, North Botswana, South Africa and some of the Arabian countries such as Saudi Arabia and Egypt. The main objective of these studies was to analyze metals such as lead (Pb), cadmium (Cd), Manganese (Mn), mercury (Hg), and arsenic (As), in some of the most commonly used medicinal plants. The results of these studies showed that metals like Lead, Cadmium, Aluminum, Mercury and arsenic in the tested plants were above the set standard [17-20].

In this study we performed a quality check of the microbial and heavy metal contamination of some selected herbs that are widely used among the Palestinian population. The study will reflect the actual situation of the herbal medicine in Palestine and will raise the herbal contamination issue with the concerned authorities. The results of this study will come up with recommendations and advice to the concerned authorities to take the necessary corrective actions.

2 Material and Methods

2.1 Chemical and Reagents:

All the reagents used were purchased from reliable resources, the following reagents were used throughout the research project: Nitric Acid 70% (Riedel-de-Haen™; Germany), Perchloric Acid 70% – 72% (Riedel-de-Haen™; Germany) were used in acid digestion of herbals, Tryptic Soy Agar (Difco™; France), Sabouraud Dextrose Agar (Difco™; France) were used in media preparation, Tryptic Soy Broth (Difco™; France), Tween80 (Difco™; France) were used in Fluid 3 preparation used in the microbiological testing.

2.2 Instrumentations

Hotplate (Model LMS-1003; Lab Tech) was used in acid digesting of the collected herbs. An Atomic Absorption Spectrometer (Model ICE3000; Thermo Scientific) was used in the quantitative analysis testing of heavy metals. Autoclave (Model DLOV 3764; DE-Lama) was used in sterilization of fluid 3 (F3) and Ager. Laminar Air Flow (BBS-V1300; Biobase), Incubators (BC3100-R1; Biorold) were used in microbiological testing of herbal samples, and a Vortex (Genie 2 SI) was used for sample mixing.

2.3 Plant Collection

Five medicinal plants that are widely used in Palestine were collected from popular herbal selling stores and from community pharmacies which sell traditional herbal medicine, these plants include: *H. sabdariffa* (Roselle), *P.
anisum (Anise), M. chamomilla (Chamomile), C. azorolus (Hawthorn), and Zingiber officinale (Ginger). Botanical characterization was conducted at An-Najah National University in the Pharmacognosy Laboratory and kept under the herbarium voucher specimen numbers: Pharm-PCT-1195, Pharm-PCT-2768, Pharm-PCT-178, Pharm-PCT-712, and Pharm-PCT-2724.

The sources of the selected plants were from different places, for example: Hawthorn is a Palestinian native plant, so all of the collected samples were harvested from Palestine. Anise was imported from south Asian countries, Roselle and Chamomile were imported from Egypt.

All the samples were grinded to fine particles to a powder form in our research lab. They were put in a glass container and were stored in a dry, dark and cool place during the project period. The total numbers of the herbal samples collected and analyzed were nineteen samples.

2.4 Elemental analysis

2.4.1 Acid Digestion of the herbal sample

Acid Digest was prepared by oxidizing 0.2 g of the dry powder plant with 10 ml of an acid mixture of (2:1) Nitric acid: Perchloric acid and the powdered plants were stirred with the mixture for overnight. The mixture was then filtered; 1 ml of the filtrate was diluted to 20 ml of distilled water. The diluted samples were used in the quantitative measurement of Lead (Pb), Cadmium (Cd), Cupper (Cu) and Zinc (Zn) by using Atomic Absorption Spectrophotometer. The analysis was performed in triplicate for every herbal sample [21].

2.4.2 Elemental quantitative analysis

Three point standard concentrations were prepared for each metal to be analyzed. The samples were then diluted with water to be within the calibration curve range. The concentrations of the samples were determined using the calibration curve regression equation [22]. The three-point calibration concentration for each measured metal is illustrated in Table 1

| Metal (ppm) | Zinc (Zn) | Cadmium (Cd) | Lead (Pb) | Cupper (Cu) |
|-------------|-----------|--------------|-----------|-------------|
| Standard #1 | 0.100     | 0.050        | 0.250     | 0.100       |
| Standard #2 | 0.300     | 0.100        | 0.500     | 0.300       |
| Standard #3 | 0.500     | 1.000        | 1.000     | 0.500       |

2.5 Microbiological testing

Bacterial and fungal contamination were tested using procedures described in the United State Pharmacopoeia-USP [23].

2.5.1 Media Preparations

Fluid 3 “F3” preparation: Fluid 3 was prepared by adding 15 g Tryptic Soy Broth in 500 ml distilled water then 1 ml of Tween 80 was added. Water was added gradually to a final volume of 1000 ml.

Tryptic Soy Ager Preparations: This media was prepared by suspending 40 g of the powder in 1 L of Purified water. The mixture was thoroughly stirred with heat and was boiled for one minute to dissolve the powder completely. This media was used for bacterial growth.

Sabouraud Dextrose Ager Preparation: This media was prepared by dissolving 65g of the powder in 1 L of purified water, mixed thoroughly with heat and frequent agitation to dissolve the powder completely. The agar media was autoclaved by heating it at 121°C for 15 minutes. This media was used for cultivation of fungi. The media was put it in the oven at 50°C to keep it liquid and to use it when needed.

2.5.2 Sample transplantation and incubation

A dry herbal sample (3 g) was added to Fluid 3 (30 g). Serial dilutions of the samples were prepared: (10^{-1}), (10^{-2}), (10^{-3}) and (10^{-4}). Each dilution was inoculated at Tryptic Soy Ager plates which were incubated at (30–35°C) for 48 hours for bacterial identification. Fungal microbial testing was performed by inoculating the prepared dilution on
were selling a similarly low quality of herbs. This may be attributed to the improper storage conditions in all herbal shops and could be due to importing a low quality of herbs from international suppliers. The concerned authorities have to take an active role to approve and license for an imported herbal medicine before it reaches the final customer.

### Table 2: The result of heavy metals

| Metal (limit) | Result | Frequency (%) | Average (ppm) |
|---------------|--------|---------------|---------------|
| Cu (20 ppm)   | Fail   | 19 (100.0)    | 56.52         |
|               | Pass   | 0 (0)         |               |
| Cd (0.3 ppm)  | Fail   | 19 (100.0)    | 64.01         |
|               | Pass   | 0 (0)         |               |
| Zn (50 ppm)   | Fail   | 15 (78.9)     | 162.76        |
|               | Pass   | 4 (21.1)      |               |
| Pb (10 ppm)   | Fail   | 0 (0)         | 0.3210        |
|               | Pass   | 19 (100.0)    |               |

### Table 3: Zinc result in different plants

| Plant          | Total |
|----------------|-------|
| Hawthorn       | 3     |
| Chamomile      | 4     |
| Roselle        | 4     |
| Anise          | 4     |
| Ginger         | 4     |

Contamination of herbs by toxic metals can be attributed to many causes including: environmental pollution, soil composition and using fertilizers [24, 25]. Some of the metals such as Cu, Cd and Zn had a higher concentration than the allowable level, this can be attributed to the use of fertilizers & pesticides which contain these elements in their compositions [26, 27]. By contrast, for the last few years manufacturers of fertilizers have not used Pb; thus, the concentration of Pb in the soil dropped sharply in the last years [28]. Moreover, the reduced use of pewter and the gradual decrease of lead solder in cans and the replacement of lead water pipes carrying water in cities and towns has led to less lead contamination in soil & water [29, 30].

### 3.3 Microbiological testing

The microbiological result showed that the bacterial count was over the limit in 63.2 percent of the tested plants and yeast was over the allowable limit in 89.5 percent of the
total tested plants. The colony counts in the majority of the failed herbals were too many to count (TMC). The detailed microbiological results are illustrated in (Figure 1).

We further investigated if the bacterial results were significantly different for the tested plants. The Chi square statistical test was performed and the bacterial results revealed that there was no significant difference between the plants (p=0.83). However, the bacterial contamination was significantly different among the selling stores (p=0.03). The above results demonstrate that the variation in bacterial results among the selling stores could be due to their difference in handling and storage practices of the herbs they sold (Figure 2).

4 Conclusion

Herbal medicines used in the Palestinian markets don’t meet international requirements. Urgent action has to be taken by the responsible authorities such as implementing importation and registration requirements. The sellers of the herbal plants must undergo regular quality checks. The results revealed a variation among the herbal sellers especially in microbiological contamination which may be due to a difference in handling and storage practice. An awareness program for both sellers and consumers about the danger of using contaminated herbs must be implemented. Pharmacists and community pharmacies must take an important role in selling and advising the consumers about quality, storage and medical effects of the sold plants.

Conflict of interest: There Authors state no conflict of interest

Ethical approval: The conducted research is not related to either human or animals use.

References

[1] Benzie IF, Wachtel-Galor S. Herbal medicine: biomolecular and clinical aspects. 2nd edition. ed: CRC Press; 2011.
[2] IFF B, S W-G. Herbal Medicine: Biomolecular and Clinical Aspects. second ed: CRC Press/Taylor & Francis; 2011.
[3] Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: an ethical analysis. Bulletin of the World Health Organization 2008;86:594-9.
[4] Jaradat NA. Medical plants utilized in Palestinian folk medicine for treatment of diabetes mellitus and cardiac diseases. J Al-Aqsa Univ 2005;9:1-28.
[5] WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. 2007.
[6] Traditional Medicine Strategy (2002-2005), World Health Organization (WHO), Geneva, Switzerland. 2002.

[7] Ali-Shtayeh M, Jamous R, Abu-Zeitoun S. BERC 2014 "National list of Medicinal Plants in Palestine-West Bank and Gaza Strip. Til, Nablus: Biodiversity and Environmental Research Center (BERC) 2014.

[8] Langmead L, Rampton DS. Review article: herbal treatment in gastrointestinal and liver disease--benefits and dangers. Aliment Pharmacol Ther 2001;15:1239-52.

[9] Veluswamy SK, Babu AS, Sundar LM. Complementary Role of Herbal Medicine and Exercise in Cardiovascular Disease Prevention and Management: A Review of Evidence. Curr Pharm Des 2017;23:1253-64.

[10] Garcia-Alvarez A, Mila-Villarroel R, Ribas-Barba L, Egan B, Badea M, Maggi FM, et al. Usage of Plant Food Supplements (PFS) for weight control in six European countries: results from the PlantLIBRA PFS Consumer Survey 2011-2012: BMC Complement Altern Med. 2016;16:254. doi:10.1186/s12906-016-1227-5.

[11] Srivastava JK, Shankar E, Gupta S. Chamomile: A herbal medicine of the past with bright future: Mol Med Report. 2010 Nov 1;3(6):895-901. doi:10.3892/mmr.2010.377.

[12] Shojaii A, Abdollahi Fard M. Review of Pharmacological Properties and Chemical Constituents of Pimpinella anisum: ISRN Pharm. 2012;2012:510795. doi:10.5402/2012/510795.

[13] Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food Chem Toxicol 2008;46:409-20.

[14] Abu-Gharbieh E, Shehab NG. Therapeutic potentials of Crataegus azarolus var. eu-azarolus Maire leaves and its isolated compounds: BMC Complement Altern Med. 2017;17:218. doi:10.1186/s12906-017-1729-9.

[15] Guardiola S, Mach N. [Therapeutic potential of Hibiscus sabdariffa: a review of the scientific evidence]. Endocrinol Nutr 2014;61:274-95.

[16] Instructions for Registration and Trading of Medicinal Plants and Food Supplements. Palestine: Ministry of Health; 2018.

[17] Annan K, Kojo AI, Cindy A, Samuel AN, Tunkumgnen BM. Profile of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations: Pharmacognosy Res. 2010 Jan-Feb;2(1):41-4. doi:10.4103/0974-8490.60579.

[18] Abou-Arab AA, Abou Donia MA. Heavy metals in Egyptian spices and medicinal plants and the effect of processing on their levels. J Agric Food Chem 2000;48:2300-4.

[19] Abba D, Inabo H, Yakubu SE, Olonitola OS. Contamination of Herbal Medicinal Products Marketed in Kaduna Metropolis with Selected Pathogenic Bacteria: Afr J Tradit Complement Altern Med. 2009;6(1):70-7.

[20] Hitokoto H, Morozumi S, Wauke T, Sakai S, Kurata H. Fungal contamination and mycotoxin detection of powdered herbal drugs: Appl Environ Microbiol. 1978 Aug;36(2):252-6.

[21] Annan K, Dickson RA, Amponsah IK, Nooni IK. The heavy metal contents of some selected medicinal plants sampled from different geographical locations. Pharmacognosy Research 2013;5:103-8.

[22] Abualhasan M, Basim A, Salahat S, Sofan S, Al-Atrash M. Quality of water used in Palestinian hemodialysis centers. Public Health 2018;165:136-41.

[23] United State Pharmacopoeia. 2017.

[24] Boros-Lajszner E, Wyszkowska J, Kucharski J. Use of zeolite to neutralise nickel in a soil environment. Environmental Monitoring and Assessment 2017;190:54.

[25] Ahmad IZ, Ahmad A, Mabood A, Sofan S, Al-Atrash M. Effects of Different Metal Stresses on the Antioxidant Defense Systems of Medicinal Plants. In: Khan MIR, Khan NA, editors. Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress. Singapore: Springer Singapore; 2017. p. 215-56.

[26] Mortvedt J. Heavy metal contaminants in inorganic and organic fertilizers. Fertilizers and Environment: Springer; 1996. p. 5-11.

[27] Biling W, Zhengmiao X, Jianjun C, Jiang J, Qiufeng S. Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (Brassica chinensis L.) in a mining tailing contaminated soil. Journal of Environmental Sciences 2008;20:1109-17.

[28] Pieper KJ, Tang M, Edwards MA. Flint Water Crisis Caused By Interrupted Corrosion Control: Investigating “Ground Zero” Home. Environmental Science & Technology 2017;51:2007-14.

[29] Pieper KJ, Nystrom VE, Parks J, Jennings K, Faircloth H, Morgan JB, et al. Elevated Lead in Water of Private Wells Poses Health Risks: Case Study in Macon County, North Carolina. Environmental Science & Technology 2018;52:4350-7.

[30] Olson TM, Wax M, Yonts J, Heidecorn K, Haig S-J, Yeoman D, et al. Forensic Estimates of Lead Release from Lead Service Lines during the Water Crisis in Flint, Michigan. Environmental Science & Technology Letters 2017;4:356-61.