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Microbial assessment of some medicinal herbs sold in Makkah, Saudi Arabia

Basam H. Mashat[1], Shawkat M.Fathi*[2,3] and Ehab S. Elkhayat[4]

[1]Dept. of Environmental and Health studies, The Custodian of the Two Holy Mosques Institute for Hajj and Omra Research, Umm Al-Qura University, KSA
[2]Dept. of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, 51452 Buriadh, Saudi Arabia
[3]Food Hygiene Dept., Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt.
[4]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, Egypt

Abstract : Forty samples of ten of the most commonly sold medicinal herbs, either packed or unpacked, collected from herbal stores located in different areas in Makkah, Saudi Arabia during Hajj season 1435H\2014 for microbial assessment. The average of total aerobic colony, *Enterobacteriaceae* and mold and yeast counts were 600/g and 9500/g in packed *Olea europaea* and *Melissa officinalis* and 900/g and 5000/g in unpacked *Matricaria chamomila* and *Origanum majorana*, 1000/g and 6600/g in packed *Cassia acutifolia*and *Melissa officinalis* and 180/g and 3400/g in unpacked *Trachyspermum copticum* and *Thymus vulgaris*, 1200/g and 7700/g in packed *Salvia officinalis* and 500/g and 3400/g in unpacked *Origanum majorana*, respectively. Some microorganisms of public health impact such as *Salmonella* and *Escherichia coli* were isolated from some samples, in addition to several molds which were also identified. Results were discussed and compared with international microbial quality standards of medicinal herbs.
Key words : Assessment, Microbial contamination, Medicinal herbs, Standards.

Introduction

Traditional herbal medicine is widely practiced nowadays. The quality assessment of these herbs and herbal formulations is very important in order to justify their incorporation in modern treatment approaches. World Health Organization estimated that, 80% of the populations of most countries of the world rely on herbs or indigenous forms of medicine as a part of the primary healthcare [1]. Medicinal plants and their natural products are used to prevent or treat a variety of diseases [2-4]. Recent trends in medicine have been associated with public interest in nations to use natural methods for prevention and treatment of diseases [5-7]. Medicinal plants provided the lead molecules for semi-synthetic compounds or traditional forms of drug dosage forms including herbal extracts, essential oils, … etc [8-10].

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Herbal preparations normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may represents additional contamination sources, as may be the case with *Escherichia coli* or *Salmonella* spp. High percentage of bacteria and fungi are from naturally occurring microflora, aerobic spore-forming bacteria that frequently predominate [11]. Many medicinal herbs have contamination potential with different microorganisms due to contaminated raw materials and unhygienic production conditions. They are highly contaminated with pathogenic bacterial flora due to poor quality control and preparation standards[12]. It is thus mandatory to apply microbiological limit tests of herbal medicinal preparations to ensure that the product is free from any latent risk. Microbial growth occurs during harvesting, handling and production, transportation, packaging and storage. Plant soil transfers lots of bacteria and fungi to the plant materials. Aerobic sporulating bacteria frequently predominate in this to which additional contamination and microbial growth occur during harvesting, handling and production [13].

Regarding microbial evaluation of medicinal herbs, Osei-Adjei, *et al*. (2013) [14], reported that, more or less all the decoctions were contaminated with aerobic bacteria and/or fungi. Among sixteen samples tested, there were three samples showed microbial counts greater than $1.0x10^9$cfu/ml, and one sample showed the lowest aerobic bacterial count of $1.0x10^2$cfu/ml. Fungal contaminations were found in thirteen (81.3%) of the samples, one sample showed the highest contamination of $3.2x10^5$cfu/ml. However, Rajapandiyan, *et al.* (2013), reported that total aerobic count of the most marketed herbal drugs had bacteriological limit exceeding WHO limit[12]. Isolation and identification of microbes showed that the samples were contaminated with more than one bacterial pathogens such as *S. aureus*, *E. coli*, *P. aeuroginosa*, *Shigella* sp and *Salmonella* spp. Whereas, Namdari, *et al.* (2014), [15] purchased randomly 64 samples of eight type of herbal extracts retailed in Isfahan markets for microbial analysis according to Iranian national standard protocols. The total bacterial count, including contamination with coliforms, sulphite reducing *Clostridium*, *Entrococci*, *Pseudomonas aeroginosa*. They found that 37% of the samples were unacceptable and not consumable. On the other side, a wide spectrum of fungi including *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Rhizopus* and *Mucor* species were isolated from Croatian herbal teas and medicinal plants [16]. However, examination of the microbial quality of mint has shown that the most abundant fungi were *Fusarium* and *Verticillium* [17,18].

Herbal products usually contain bacteria and molds from soil and atmosphere, but there are acceptable limits of contamination. For example, the European Pharmacopoeial limits of bacterial contamination are: 10^5cfu/g for total aerobic bacteria, 10^3cfu/g for enterobacteria and other Gram negative organisms. The pathogenic enterobacteriacea such as *Escherichia coli* and *Salmonella* should always be absent [19,20]. Furthermore, American Herbal Products Association (AHPA) (2016) [21], determined the microbial limits for botanical ingredients in colony-forming units/gram (cfu/g) for dried unprocessed herbs for use as ingredients in dietary supplements as 10^7, 10^5 and 10^4 for total aerobic microbial count, total combined yeast and mold count and enterobacterial count (bile-tolerant Gram-negative bacteria), respectively. Moreover, *Escherichia coli* and *Salmonella* spp. should be not detected in 10 and 25 g of dried unprocessed herbs, respectively.

The present study was designed to evaluate the microbial quality of some common used medicinal herbs marketed in Makkah during Hajj season 1435H \ 2015, and comparing results with standards and regulations of microbial limits to assess such herbs from public health point of view.

Experimental:

Collection of samples:

A total of 40 samples of 10 herbal items (4 samples of each item) of the most commonly used medicinal herbs were purchased randomly from 8 different herbal stores distributed in different areas in Makkah, Saudi Arabia in order to evaluate their microbial quality (Table 1). The collected medicinal herbal samples are classified into two groups either packed by the licensed manufacturer or unpacked (bulk) which is sold by weight as costumer request (Fig. 1 and 2).

Table 1: Geographical source of the collected medicinal herbs

Medicinal herbs	**Packed**	**Unpacked**
Cassia acutifolia	India	Saudi Arabia
Hibiscus sabdarifa	Sudan	Sudan
Matricaria chamomila	Egypt	Egypt
Melissa officinalis	Syria and Jordan	Syria and Jordan
Nigella sativa	India	Saudi Arabia
Olea europaea	Jordan and Syria	Jordan and Saudi Arabia
Origanum majorana	India	India
Salvia officinalis	Syria and Sudi Arabia	Sudi Arabia
Trachyspermum copticum	India	India
Thymus vulgaris	Jordan and Syria	Jordan and Syria

Figure 1: Packed medicinal herbs ready for sale at a herbal store in Makkah, Saudi Arabia

Figure 2: Unpacked bulk medicinal herbs for sale by weight in another herbal store in Makkah, Saudi Arabia

Preparation of samples:

Ten grams of each collected sample were transferred into a sterile Stomacher's bag containing 90 ml of peptone water and thoroughly mixed by Stomacher to obtain 1/10 dilution, subsequently 10 fold serial dilutions were prepared with peptone water up to 10^{-5} from the original dilution according to American Public Health Association (APHA, 2014) [22].

Aerobic plate count:

Duplicate sterile Petri-dishes were inoculated with 1 ml from each dilution by automatic pipettes, then 15 ml of sterilized agar cooled to 45°C and poured into Petri-dishes. The inoculated plates, after being

thoroughly mixed were incubated at 35 °C for 48 ± 2 hours before examination according to official methods of analysis, (AOAC., 1995) [23].

Enterobacteriaceae count:

Aliquots of 1ml of each previously prepared dilutions delivered into duplicate sets of Petri-dishes. Then 10 ml of Violet-Red Bile Glucose agar, melted and tempered to 45°C were poured into the Petri-dishes and mixed Immediately. After solidification, a cover layer of 10 ml of Violet- Red Bile Glucose agar were poured over plate contents. Plates were incubated at 35 - 37 °C for 21 ± 3 hours. All purple colonies were counted and reported as *Enterobacteriaceae* count per gram of sample [21].

Mold and yeast count:

Potato dextrose agar was used in a similar manner as previously described for enumeration of aerobic plate count. The plates were incubated at 20 °C for 5 days. The colonies for each mold and yeast were enumerated separately and recorded according to APHA (2014) [22].

Isolation of pathogenic microorganism:

Some pathogenic microorganisms such as *Salmonella* spp and *Escherichia coli* were isolated by conventional methods as recommended by United States Pharmacopeia (USP) 2011 [24].

Results and Discussion :

Previous investigations pointed out that medicinal plant materials normally carry a large number of microbes originating from the soil while various kinds normally adhere to leaves, stem, flowers, seeds, and roots. Additionally, contaminants may be introduced during harvesting, handling, and production of various herbal remedies [25], as well as the use of untreated water and exposure to the environment including dust and other particulate matter were the two most likely sources of medicinal herbs contamination during their preparations [26].

Table 2: Average of total aerobic plate, Enterobacteriaceae, mold and yeast counts/g of packed medicinal herbs

Medicinal herbs	Aerobic plate counts	*Enterobacteriaceae*	Mold	Yeast	Total mold and yeast
Matricaria chamomila	5600	0	100	100	200
Cassia acutifolia	3000	1000	0	0	0
Salvia officinalis	1000	3000	1200	7700	8900
Melissa officinalis	9500	6600	500	400	900
Hibiscus sabdarifa	6300	0	0	0	0
Olea europaea	600	0	0	0	0

Table 3: Average of total aerobic plate, Enterobacteriaceae, mold and yeast counts/g of unpacked medicinal herbs

Medicinal herbs	Aerobic plate counts	*Enterobacteriaceae*	Mold	Yeast	Total mold and yeast
Matricaria chamomila	900	0	0	0	0
Origanum majorana	3900	0	500	3400	3900
Nigella sativa	1500	0	400	0	400
Trachyspermum copticum	1300	180	200	0	200
Thymus vulgaris	3000	3400	500	0	500

Table 4: Pathogenic microorganisms isolated from packed medicinal herbs

Medicinal herbs	***Salmonella* spp.**	***E. coli***	**Identified fungi**
Matricaria chamomila	-ve	-ve	*Penicillium* spp
Cassia acutifolia	-ve	-ve	-
Salvia officinalis	+ve	-ve	*Aspergillus niger* *Aspergillus flavus*
Melissa officinalis	-ve	Atypical	*Aspergillus niger* *Aspergillus flavus* *Penicillium* spp
Hibiscus sabdarifa	-ve	-ve	-
Olea europaea	-ve	-ve	-

Table 5: Pathogenic microorganisms isolated from unpacked medicinal herbs

Medicinal herbs	***Salmonella* spp.**	***E. coli***	**Identified fungi**
Matricaria chamomila	-ve	Typical	*Alternaria* spp
Origanum majorana	+ve	Typical	*Aspergillus niger* *Alternaria* spp
Nigella sativa	+ve	Typical	-
Trachyspermum copticum	-ve	Atypical	-
Thymus vulgaris	-ve	-ve	-

Figure 3: Isolated Aspergillus niger from packed Salvia officinalis

Figure 4: Isolated Penicillium spp from packed Melissa officinalis

Although the obtained results revealed the presence of microbial contaminants in all of the investigated medicinal herbs samples , but still not exceeding the acceptable limits of microbial count as reported by WHO (2007) [20] and AHPA (2016) [21] which demonstrated that total aerobic microbial count should not exceed 10^5 and 10^7cfu/g, respectively.

As illustrated in tables (2) and (3), 50% and 60% of the examined packed and unpacked medicinal herbs samples were contaminated by *Enterobacteriaceae*, respectively. Also, 50% and 20% of the investigated packed and unpacked samples were harboring mold and yeast. The enumeration of both *Enterobacteriaceae* and total mold and yeast in contaminated samples were below the permissible limits determined by WHO and AHPA. The packed samples (12), were contaminated by *Enterobacteriaceae* more than the unpacked samples (10), which indicated contamination of the herbs before packaging or at the packaging place. On the other hand, the unpacked herbal samples were more contaminated by mold, which indicated the role of packaging in preventing mold contamination.

Medicinal herbs may be associated with a variety of microbial contaminants which depends on several environmental factors and exerts an important impact on the overall quality of herbal products and their preparations. Microbial contamination originates from primary and secondary sources. The primary contamination is the naturally occurring microbial flora of the plant to be harvested. Secondary contamination is caused by handling of the plant material (human intervention, equipment, buildings, air ventilation systems, and contamination during transportation). Minimizing contamination with micro-organisms and microbial toxins should be ensured by monitoring and limiting both primary and secondary contaminations, i.e. by prevention rather than by use of decontamination methods. Since the safety, effectiveness, and quality of finished herbal medicinal products depend on the quality of their raw materials and how these materials were handled through production processes, the Ministry of Health should adopt active documentation and training of herbalist to sanitize their practices. Post-marketing surveillance, pharmacovigilance, and random screening of herbal products and herbal stores should be entrenched in the regulatory framework to quickly dictate any possible adverse effect and to ensure the consistency and quality of distributed herbal medicines [27].

Regarding the isolated pathogenic microorganisms from the examined medicinal herbs samples, *Salmonella* spp. were found in packed *Salvia officinalis* and unpacked *Origanum majorana* and *Nigella sativa*. However, typical *E. coli* were isolated from *Matricaria chamomile*, *Origanum majorana* and *Nigella sativa* as shown in tables (4) and (5). *Salmonella* and *Shigella* species should not be present in herbal preparations intended for internal use, at any stage. Other microorganisms should be tested for and should comply with limits set out in regional, national or international pharmacopoeias [20].

The presence of the *Aspergillus niger* and *A. flavus* isolated from the examined herbal samples has the potential for toxin production in the products and constitute public health significance. Therefore, strict Good Manufacturing Practices and hygienic practices should be followed in order to minimize added contamination. Also, raw materials of good microbial quality should be used in the production of these medicines. As well as, the need for regulatory measures to ensure their safety, efficacy and quality is therefore imperative.

Regulation of herbal products varies from country to country have great impact on microbial count of herbal products, where in some countries, herbal remedies are well-established, whereas in others they are regarded as food and therapeutic claims are not allowed. There are many traditionally used herbs and so much folk knowledge about these herbal medicines in developing countries such as Nigeria, but scientific evidence from tests done to check their safety and effectiveness are either lacking or limited. However, there are weak legislations and loose regulatory environment which have been taken advantage of by commercial vendors of herbal therapies. The public is at great risk in unregulated commercial promotion and sale of herbal remedies. The vulnerability of the consuming public is made worse by the general, but wrong perception of herbal medicines as entirely natural and safe [27-29]. Therefore, There is urgent need for practitioners to be educated on the hygienic preparation, storage and dispensing of herbal medicines to prevent microbial contamination [26].

In order to improve and grantee the quality of herbal extracts, good observations based on hygienic conditions and applying good manufacturing practices, good harvesting practices, safe handling and storage during preparation and distribution had to be stressed and guaranteed during planting, harvesting, shipment, packaging and retailing.

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