



# Microbial indices of industrial and traditional medicinal herbs in Ahvaz, Iran

Abdolghani Ameri<sup>1</sup>, Maryam Ekhtelat<sup>2,\*</sup>, Sara Shamsaei<sup>1</sup>

<sup>1</sup> Department of Drug and Food Control, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>2</sup> Medicinal Plant Research Center, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

\*e-mail: [ekhtelat-m@ajums.ac.ir](mailto:ekhtelat-m@ajums.ac.ir), [maryam\\_ekhtelat@yahoo.com](mailto:maryam_ekhtelat@yahoo.com)

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## Abstract:

**Introduction.** Medicinal herbs are susceptible to microbial contamination which can have profound effects on the consumer's health. Our study aimed to evaluate microbial contamination of common medicinal herbs in Ahvaz.

**Study objects and methods.** We collected 80 samples of traditional and industrial medicinal plants from the supply market, namely valeriana, fennel, licorice, and shirazi thyme. The reference method was used to determine microbial indices such as the total count of microorganisms, yeast and mold, *Bacillus cereus*, coliforms, and *Escherichia coli*.

**Results and discussion.** We found that the total microbial count, yeast and mold, *B. cereus*, and coliform contamination accounted for 45, 77, 55, and 55% of the total samples, respectively, exceeding the allowed limits. There was a significant difference between the industrial and traditional samples in fungal and coliform contamination, with the traditional samples being more highly contaminated. However, no significant difference was observed between them in total count and *B. cereus* contamination. *E. coli* contamination was detected in 31.2% of the samples, mostly in traditional. Total microbial count and yeast and mold contamination were highest among valeriana plants. Fennel showed the highest *B. cereus* and coliform contamination. The lowest contamination was observed in licorice.

**Conclusion.** The results showed that a considerable percentage of the medicinal herbs under study were contaminated at levels exceeding the standard limits. Plants could be contaminated during harvesting, processing or storage. Finally, different species of plants have different antimicrobial activities that affect their microbial contamination.

**Keywords:** Microbiology, microbial contamination, quality control, medicinal herbs, total microbial count

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## INTRODUCTION

Medicinal plants become contaminated by a variety of sources such as heavy metals, insect larvae and seed, different bacteria, and fungi [1]. Heat and humidity of the environment, long-time drying, irrigation with contaminated water, and lack of farmer training may result in considerable microbial contaminations and reduce the quality of plants. Moreover, microbial contamination of plants may take place during unhealthy collection, cleaning, storage, transportation, and packaging. Contact of herbal products with external factors such as plastic, glass, and other materials may lead to cross contamination.

Medicinal plants can be contaminated by a wide range of microorganisms, such as fungi, yeasts, protozoa, and viruses, most of which are transferred from soil [2, 3]. Total microbial count is an important factor in determining the health status or probable detection of a contamination source [4]. Yeast and mold are the most common contaminants of medicinal herbs. Various species of molds and yeasts that proliferate on food stuff secrete metabolic toxic materials such as mycotoxins, which are harmful for humans and animals [5]. The WHO (World Health Organization) has a large amount of data in this direction [6].

Coliforms (*Escherichia*, *Enterobacter*, and *Klebsiella*) from the *Enterobacteriaceae* family inhabit human and animal intestines. Most of them are not pathogenic, although some *E. coli* strains could be highly pathogenic and cause food poisoning [7]. According to Iran's national standard, coliform contamination in most dried vegetables should not exceed the maximum level of 1000 CFU/g, while the presence of *E. coli* is not allowed<sup>I</sup>. *Bacillus cereus* is widely distributed in the environment and some its strains are harmful for human health and can cause food poisoning. This bacterium secretes enterotoxin, hemolysin, and lecithinase C which are responsible for disease [9]. Dried vegetables contamination by *Bacillus cereus* should not exceed the maximum level of 100 CFU/g.

Valeriana (*Nardostachys jatamansi* L.) from the *Valerianaceae* family is known for its anticonvulsant, sedative, anti-asthmatic, and cardiotoxic properties [9]. Fennel (*Foeniculum vulgare* L.) from the Umbelliferae family has culinary and medicinal properties (anti-inflammatory, anti-spasmodic, diuretic, laxative, analgesic, antioxidant, and wound-healing) [10]. Licorice (*Glycyrrhiza glabra* L.) growing in Mediterranean countries, Central Asia, and Europe has a wide range of pharmacological effects such as antioxidant activity, liver protection, and regulation of the immune system [11]. Shirazi thyme (*Zataria multiflora* Boiss L.) is used in the south of Asia as tea or spice and in traditional medicine as a gastrointestinal disinfectant, diuretic, or an anti-inflammatory remedy [12].

Contaminants such as microorganisms, heavy metals, and pesticides affect the quality and the efficacy of herbal products. Since it is impossible to remove all contaminants, precautionary measures should be taken to prevent or limit contamination [2, 3]. Therefore, our study aimed to show the effect of these contaminations on consumer's health.

## STUDY OBJECTS AND METHODS

**Collection and preparation of samples.** For this study, samples were randomly collected from medicinal herb retailers and drugstores of Ahvaz (Iran) from December 2017 for 6 months. A total of 80 samples were used: 40 traditional (10 samples for each traditional herb) and 40 industrial herbs from different companies (19 shirazi thyme, 6 fennel, 8 valeriana, and 7 licorice samples). The amounts of industrial samples were not equal due to their insufficient availability.

**Total microbial count.** Total microbial count was performed as described by Standard No. 5272, Iran<sup>II</sup>. Different dilutions of medicinal herbs were prepared and

cultured on Plate count agar (PCA, Merck, Germany). Triplicate plates for each dilution were cultured and incubated for 72 h at 30°C. Then, the average of counted colonies was measured taking into account the dilution coefficient.

**Mold and yeast count.** Fungal count was performed according to Standard No. 10899, Iran<sup>III</sup>. Different dilutions of medicinal herbs were inoculated on Sabouraud dextrose agar (SDA, Merck, Germany) in triplicates and incubated at 25°C for 5 days. Then, the average number of molds and yeasts per gram of herb was estimated.

***Bacillus cereus* detection.** To detect and count *B. cereus* (Standard No. 2324, Iran), dilutions of medicinal herbs were prepared and cultured in triplicate on Mannitol-egg yolk-polymyxin (MYP) agar (Merck, Germany) at 30°C for 48 h. The agar contained an egg yolk emulsion and polymyxin B sulfate (Shijiazhuang Pharma, China)<sup>IV</sup>. Large and pink colonies (lack of manitol fermentation) with a sedimentary halo (lecithinase producer) were counted as probable *B. cereus*. To confirm the suspected colonies, a hemolysis test was performed on Blood agar (Merck, Germany).

**Coliform detection and enumeration.** Coliform detection and enumeration were performed according to Standard No. 9263, Iran<sup>V</sup>. Different dilutions of medicinal herbs were inoculated (pour plate and two-layer culture) in triplicate on Crystal violet neutral red bile lactose (VRBL, Merck, Germany) agar and incubated at 37°C for 24 h. Typical red purple colonies were confirmed on Brilliant green bile lactose (BGBL) broth (Merck, Germany) contained in Durham tubes at two temperatures (37 and 44°C) for 24 h.

***Escherichia coli* detection.** Following coliform detection, positive BGBL tubes (gas production) were inoculated into peptone water and incubated at 44°C for 48 h. Gas production in BGBL and production of indole in peptone water were recorded for presence of *E. coli*<sup>VI</sup>.

**Statistical analysis.** Analysis of data was performed using SPSS statistical software. The significance of the results was evaluated by McNemar nonparametric test with significance level of  $P < 0.05$ .

## RESULTS AND DISCUSSION

**Total microbial contamination.** According to the results of total microbial count (Fig. 1), 45% of the total

<sup>III</sup> Standard No. 10899, Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of yeasts and molds. Institute of Standards and Industrial Research of Iran; 2008.

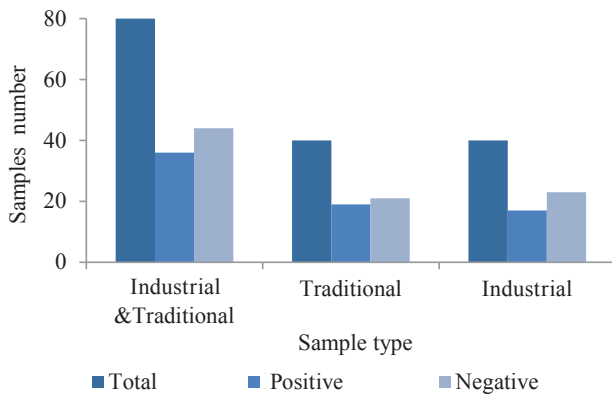
<sup>IV</sup> Standard No. 2324, Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of presumptive *Bacillus cereus*-colony count technique at 30°C. Test method. Institute of Standards and Industrial Research of Iran; 2006.

<sup>V</sup> Standard No. 9263, Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of coliforms-colony count technique. Institute of Standards and Industrial Research of Iran; 2007.

<sup>VI</sup> Standard No. 2946, Microbiology of food and animal feeding stuffs-detection and enumeration of presumptive *Escherichia coli*. Most probable number technique. Institute of Standards and Industrial Research of Iran; 2005.

<sup>I</sup> Standard No. 5939, Microbiology of dehydrated vegetables-specifications. Institute of Standards and Industrial Research of Iran; 2008.

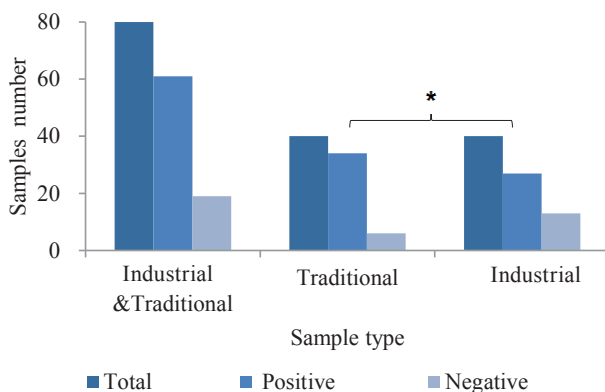
<sup>II</sup> Standard No. 5272, Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of microorganisms-colony count technique at 30°C. Institute of Standards and Industrial Research of Iran; 2007.



**Figure 1** Total microbial contamination in medicinal herbs. Total is number of samples. Positive – contamination over the limit ( $10^5$  CFU/g). Negative – contamination below the limit

herbs (36 samples out of 80) showed contamination over the limit ( $10^5$  CFU/g). Of them, 19 (48%) and 17 (43%) were traditional and industrial, respectively. The microbial load in the samples with contamination over the limit varied from  $5.03 \pm 0.03$  to  $6.25 \pm 0.03$  log CFU/g. This ranged from  $5.14 \pm 0.01$  to  $6.25 \pm 0.03$  in the traditional samples and from  $5.03 \pm 0.3$  to  $6 \pm 0.05$  log CFU/g in the industrial samples. However, there was no significant difference between the total microbial contamination in the traditional and industrial samples ( $P > 0.05$ ). Among the studied herbs, valeriana (*Nardostachys jatamansi* L.) and licorice (*Glycyrrhiza glabra* L.) showed the highest and the lowest contamination – 56 and 18%, respectively. Also, the total microbial contamination of shirazi thyme (*Zataria multiflora* Boiss L.) and fennel (*Foeniculum vulgare* L.) was over the limit in 55% and 44% of the samples, respectively.

**Fungal contamination.** The results of fungal contamination are presented in Fig. 2. As we can see, 61 (77.5%) out of 80 samples had mold and yeast contamination over the limit ( $10^3$  CFU/g).

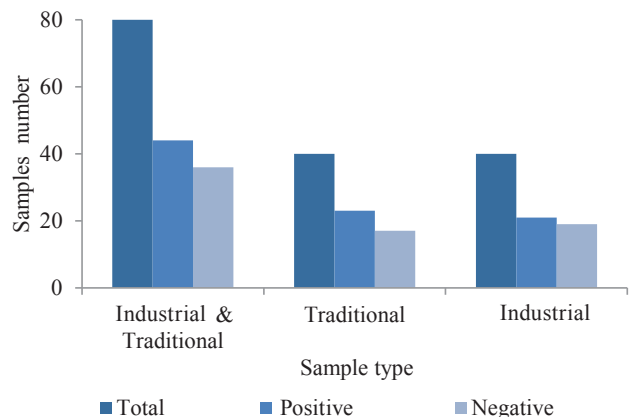


**Figure 2** Mold and yeast contamination in medicinal herbs. Total is number of samples. Positive – contamination over the limit ( $10^3$  CFU/g). Negative – contamination below the limit. \*  $P < 0.05$

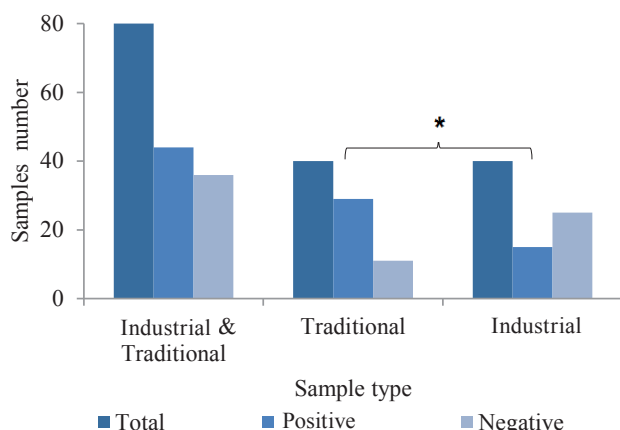
They comprised 34 (85%) traditional and 27 (67.5%) industrial samples. The fungal load in the samples with contamination over the limit varied from  $3.02 \pm 0.00$  to  $4.78 \pm 0.06$  log CFU/g. This reached from  $3.02 \pm 0.00$  to  $4.78 \pm 0.06$  in the traditional samples and from  $3.04 \pm 0.01$  to  $4.60 \pm 0.01$  log CFU/g in the industrial samples. It should be mentioned that the traditional samples were significantly contaminated with mold and yeast ( $P < 0.05$ ). Meanwhile, valeriana (100%) and licorice (47%) showed the highest and the lowest contamination, respectively. Also, 79% and 75% of shirazi thyme and fennel, respectively, showed over the limit fungal contamination.

**Bacillus cereus contamination.** *B. cereus* contamination in 44 samples (55%) of the total herbs exceeded the limit ( $10^3$  CFU/g) (Fig. 3), including 23 (57.5%) traditional and 21 (52.5%) industrial herbs. Over the limit *B. cereus* contamination varied from  $2.03 \pm 0.03$  to  $3.84 \pm 0.06$  log CFU/g. In the traditional samples, it ranged from  $2.03 \pm 0.03$  to  $3 \pm 0.06$  log CFU/g and in the industrial samples, from  $2.03 \pm 0.03$  to  $3.84 \pm 0.06$  log CFU/g. Our results showed that there was no significant difference between the traditional and industrial samples in *B. cereus* contamination ( $P > 0.05$ ). The contamination in fennel (94%) and valeriana (66%) was significantly higher ( $P < 0.05$ ). Shirazi thyme (41%) and licorice (29%) showed a lower level of *B. cereus* contamination.

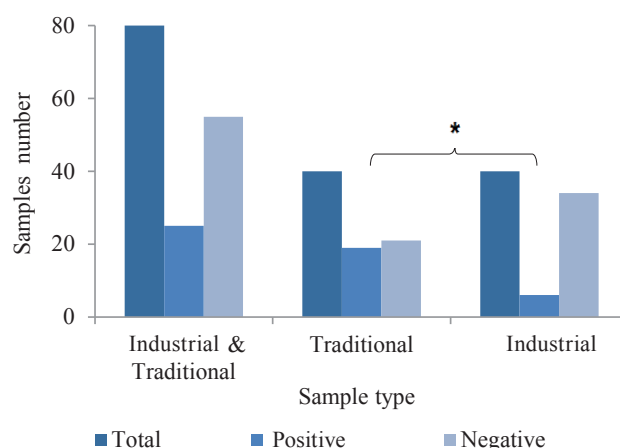
**Coliform contamination.** Coliform contamination was found over the limit ( $10^3$  CFU/g) in 44 samples (55%) of the total herbs. Among these samples, 29 (72.5%) and 15 (37.5%) were from traditional and industrial herbs, respectively (Fig. 4). Over the limit coliform contamination ranged from 3.01 to  $4.16 \pm 0.03$  log CFU/g, namely from 3.01 to  $4.16 \pm 0.03$  in the traditional samples and from  $3.03 \pm 0.02$  to  $4.15 \pm 0.03$  log CFU/g in the industrial samples. According to the results, the traditional samples showed a significantly higher coliform contamination than the industrial samples ( $P < 0.05$ ). Fennel (81%) and valeriana (66%)



**Figure 3** *Bacillus cereus* contamination in medicinal herbs. Positive – contamination over the limit ( $10^2$  CFU/g). Negative – contamination below the limit. \*  $P < 0.05$



**Figure 4** Coliform contamination in medicinal herbs. Positive – contamination over the limit ( $10^3$  CFU/g). Negative – contamination below the limit. \*  $P < 0.05$



**Figure 5** *Escherichia coli* contamination in herbs. Total is number of samples. Positive – presence of *E. coli*. Negative – absence of *E. coli*. \*  $P < 0.05$

revealed a very high level of contamination ( $P < 0.01$ ), while the lowest level was recorded in licorice (41%) and shirazi thyme (41%).

***Escherichia coli* contamination.** There should be no *E. coli* contamination in dried vegetables [8]. According to our results, 25 samples (31.2%) of the total herbs showed *E. coli* contamination. Of them, 19 (47.5%) and 6 (15%) were from traditional and industrial herbs, respectively (Fig. 5). *E. coli* contamination in the traditional samples was significantly higher than in the industrial samples ( $P < 0.05$ ). The contamination in fennel (43.7%) and valeriana (33.3%) was significantly higher ( $P < 0.05$ ) than that in licorice (23.5%) and shirazi thyme (27.5%).

One of the important aspects is contamination of medicinal herbs by different types of harmful factors such as microbes, heavy metals, as well as radioactive and chemical materials [2]. Our results, in many cases, indicated high contamination of the herbs under study with different microbial agents. These contaminations could occur during different stages of cultivation, extraction, drying, packing or distribution [2, 3, 13]. In our study, the microbial contamination level in the traditional samples was higher than that in the industrial samples. This result could be due to different production and packaging conditions.

Researchers have reported that the differences in technological level and preparation, supply and production of medicinal herbs could affect their contamination level [13–15]. Worldwide, a high level of contamination has been reported in a variety of medicinal herbs. For example, Banerjee *et al.*, in a study of 154 dried plants collected from shops in India, showed that the total microbial count was over the limit in 51% of the samples, and 97% of them had mold contamination [16].

Moreover, Abba *et al.*, in a study of powdered medicinal plants in Nigeria, reported that 87% of them had high microbial contamination [17]. Their contamination level was significantly higher than that in

our study (45%), which could be due to environmental factors, soil or inappropriate packing conditions. Some studies in different locations showed that many of the investigated medicinal herbs were contaminated with various fungi [5, 16, 18, 19]. Alwakeel, in a study on 32 samples of various medicinal plants in Saudi Arabia, showed *Bacillus cereus* as the most common microbial contaminant [20]. Martins *et al.* found the same result in more than 90% of the studied medicinal plants in Portugal [21].

In our study, fennel and valeriana showed the highest and licorice showed the lowest levels of contamination with *B. cereus* and coliform. In a study of the antimicrobial activity of Turkish spices, fennel showed a lower antibacterial effect on *B. cereus* [22]. Moreover, Lang *et al.* in Austria reported that fennel had a lower inhibitory effect on coliform than licorice [23]. It seems that the antimicrobial properties of medicinal herbs could also explain the differences in their contamination levels. In the previous studies, high microbial contaminations were reported in valeriana [19, 24]. We found that valeriana had the highest contamination level. This result can be due to the fact that most of its active medicinal ingredients are in the root of the plant, which is in direct contact with soil, so more microorganisms can be transmitted to it [19]. In our study, licorice showed a considerably lower contamination level than other plants. It could be due to differences in plant production, its active constituents, and distribution processes [3, 25]. In addition, most studies have shown that licorice had higher antimicrobial and antifungal activities than other plants, especially against *B. cereus* and *E. coli* [22, 35–38].

## CONCLUSION

In our study, we tested 80 samples of traditional and industrial herbs, such as valeriana, fennel, licorice, and shirazi thyme, for microbial contamination. The

results of the experiment showed that microbial indices in considerable percentage of the samples exceeded the standard limit. Valeriana had the highest total microbial count and yeast/mold contamination, fennel – *B. cereus* and coliforms, while licorice was not massively contaminated.

We also revealed that fungal, coliform, and *E. coli* contamination in traditional herbs was considerably higher than that in industrial samples. However, there was no significant difference between them in total microbial count and *B. cereus* contamination.

Thus, our results demonstrated the importance of monitoring medicinal plants contamination to control the quality of herbal products.

## CONTRIBUTION

Maryam Ekhtelat developed the original idea and the protocol. Abdolghani Ameri, Maryam Ekhtelat and Sara Shamsaei developed the protocol, analyzed the data, and wrote the manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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#### ORCID IDs

Abdolghani Ameri  <https://orcid.org/0000-0002-9034-2287>

Maryam Ekhtelat  <https://orcid.org/0000-0002-7274-0572>