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Microbial quality of some medicinal herbal products in Kashan, Iran

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ABSTRACT

Introduction: The use of medicinal plants has risen worldwide. In Iran, herbal waters and rose waters are of traditional medicinal products and as a result, they are widespreadly consumed. Therefore, diagnosis of microbial quality of these products is important. The aim of this study was to evaluate microbial quality of herbal extracts distributed in Kashan, Iran.

Methods: In this descriptive study, 256 samples of herbal waters and 191 samples of rose waters (total samples of 447) distributed in Kashan during 2012 to 2013 were purchased and transferred to laboratory. Then microbial tests such as total aerobic bacterial count, mold and yeast count, total *coliforms*, and detection of *Enterococcus*, *Pseudomonas* and *sulphite-reducing Clostridia* were evaluated based on national standard of Iran.

Results: Contamination with *Pseudomonas* and *Enterococcus* was observed in the herbal water samples. 196 cases (43.84%) of the total samples, 113 cases (44.15%) of the herbal waters and 83 cases (43.45%) of the rose waters were usable based on the national standard of Iran. Neither herbal waters nor rosewater samples were contaminated by *E.Coli* and *Sulphite-reducing clostridia*. Additionally, none of the rosewater samples was contaminated by *Coliforms* and *Pseudomonas*.

Conclusion: Based on the findings and due to the fact that these products are contaminated with aerobic mesophilic bacteria, mold and yeast, to minimize the risks we recommend to apply pasteurized temperature, high-quality packaging material and hygiene observance in processing time of herbal waters and rose waters.

Implication for health policy/practice/research/medical education:
The herbal products such as the ones distributed in Kashan might be contaminated with aerobic mesophilic bacteria, mold and yeast, therefore, it is suggested that manufacturers of herbal waters pay attention to the hygiene observance, Good Hygiene Practice, the use of pasteurization temperatures, observance of condition of the manufactory and appropriate packaging in the manufacturing processes to reduce secondary contamination and enhance the quality of the final product.

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Introduction

Medicinal plants are of the most valuable natural resources found in a wide range in Iran. The use of herbs to treat diseases has a long history in many countries, especially in Iran (1). Herbal waters and rose waters are used for nutritional purposes in many foodstuffs like sweets and ice creams as well as in treating rheumatic and heart pain, and strengthening the stomach. In addition, rose waters are also used in religious ceremonies and pilgrimage places (1,2).

Nowadays, the herb therapy and drugs of plant origin are increasingly used; in a way that about one-third to half of medicinal products found in the United States enjoy herbal origin (3). Besides, herbal products and lots of

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supplements are produced in the United Kingdom in a safe and risk-free form (3,4). In addition to public people, clinical microbiologists tend highly to use these drugs in the treatment of infections in that comparatively the side effects of these drugs are significantly lower than chemical drugs. In a study it was shown that consumption of medicinal plants increased 38% in 1990 and 1997, and revenue from the sale of these products was about 5.1 billion dollars in 1997 (5).

Bacteria are of the major causes of microbial infections and food borne poisoning. Over the past decade, the incidence of food borne microbial diseases in the developing and developed countries has been overwhelming, while there is no exact statistics of incidence and the occurrence of infections and food borne poisoning, especially in developing countries (6). The evaluation of existence of a variety of the microorganisms in herbal extracts is a mean to determine their hygienic status of this product. Also, pathogenic microbes such as enterobacter, enterococcus, shigella and streptococcus have been shown to grow on herbal materials (2). Therefore, of the most important evaluation of these products is the existence of coliforms indicating its fecal contamination (1).

Escherichia coli is a normal flora in the intestines of humans and many warm-blooded animals and they are reported to be 10^9 CFU/g. Thus, it was suggested as the specific indicator of fecal contamination in 1982. In microbial tests, searching for *coliforms* spp. are utilized as an important indicator of health products such as herbal extracts (7).

Enterococcus is resistant bacteria to bile salts and sodium azide and these bacteria can confirm fecal contamination of food material through the presence or absence of *Coliform*. Hence, these are used as a secondary indicator of fecal contamination in foodstuffs (8).

Regarding to the spending too much time and money in identifying different types of foodborne pathogens, identification of indicator microorganisms was carried out. As an example, *sulphite-reducing Clostridia* are anaerobic bacteria, producing spores and belong to genus *Clostridia* and *Bacillaceae* family. Spores of *sulfite-reducing Clostridia* are widely distributed in the environment. They are found in human and animal faeces as well as in sewage and soil. Unlike coliforms and *E. coli*, they remained alive in water for a long time and are more resistant against chemical and physical agents compared to vegetative forms and can indicate the presence of defects in the refining process of the products and are presented as an indicator of intermittent contamination (7,9). In a study in Birjand, the microbial contamination of herbal waters was reported to be 80% (1).

So far, a few studies on microbial and hygienic quality of herbal waters and rosewaters have been done in Iran. According to the consumption approach to this product in raw as a medicinal herbal product, this study was carried out to examine the microbial quality of herbal waters and rose waters distributed in Kashan, Iran.

Materials and Methods

Sample collection

This is a descriptive study carried out on 256 samples of herbal waters and 191 samples of rose waters (totally 447 samples) distributed in Kashan during the 2012 to 2013. Food samples were taken to Kashan University of Medical Sciences for laboratory tests. Then microbial tests were performed according to Iranian national standards with the code numbers as follow: 3270, 3545, 5272, 7724-2, 7725-1, 5353, 4207 and 8869 (10-17).

Total aerobic bacterial count

The total bacteria were counted by pour plate method in the plate count agar (Merck ink, Darmstadt, Germany). Then they were incubated for 72 h in the temperature of 30±1 °C (10).

Mold and yeast count

To count mold and yeast, 100 ml of sample passed through a sterile filters (0.22 µm) and through using a forceps, filter was placed onto the surface of the Yeast Extract Glucose Chloramphenicol Agar (YGCA; Merck Ink, Darmstadt, Germany) and then, the media were incubated at 25 °C for 3 to 5 days (11).

Detection of coliforms spp. and E.coli

In order to detect *coliforms* spp. and *E.coli*, 100 ml of the sample was passed through sterile filter (0.22 µm). The filter was placed on the surface of MacConkey agar medium (Merck ink, Darmstadt, Germany) by means of a sterile forceps. It was incubated at 37 °C for 24 to 48 h at 37 °C. In the case of colonies growth, they were counted and reported in the samples of 100 ml. The grown colonies were used for differential diagnosis of *E. coli* by TSI, SIM, MR-VP and urea Tests (Merck ink, Darmstadt, Germany) (16).

Detection of Enterococcus spp.

To detect *Enterococcus* spp., 100 ml of the sample was passed through sterile filter (0.22 µm). The filter was placed on the KF *Enterococcus* (Merck ink, Darmstadt, Germany) specific culture using sterile forceps. The plates were incubated at 37 °C for 48 h. The Formation of colonies with red, cherry and pink color demonstrated the existence of *Enterococcus* (15).

Detection of Pseudomonas

Pseudomonas presence was monitored by passing 100 ml of samples through sterile filter (0.22 µm), cultivating the filter retentive on the surface of cetrimide agar (Merck ink, Darmstadt, Germany) using sterile forceps. Then plates were incubated for 24 to 48 h at 37 °C. The formation of colonies with green color indicates the presence of

Pseudomonas (14).

Detection of sulfite-reducing Clostridia

Samples were put into water bath to monitor *sulfite-reducing Clostridia* presence. Heating at 70 °C for 20 min causes heat shock and spores transformation to vegetative form. After cooling, 100 ml of the sample was passed through a membrane filter. By means of sterile forceps, filter was placed on specific Sulfite Polymyxin Sulfadiazine culture (SPS). Then the medium was incubated in an anaerobic jar at 42 °C for 48 h. If sulfite- reducing *Clostridia*, are present in the sample, black colonies will be observed (17).

Statistical analysis

The data were analyzed using SPSS software version 11.5 through Fisher's exact test.

Results

Based on the results gained from the total sample, 43.84 percent (196 cases) of the herbal waters and rosewaters were usable according to national standards of Iran and 56.16% (251 cases) were non-usable. 108 cases (42.18%) of herbal water samples were uncountable regarding to the total count of mesophilic bacteria and total bacteria count for 116 (45.32%) cases was less than 100 cfu/ml. Also, 32 cases (12.5%) of samples had total bacterial count between 10^2 to 6 $\times10^3$ cfu/ml which had an average of $1.153\times10^3\pm1.738\times10^3$ cfu/ml. Nine cases (3.5%) of samples shown contamination combined to mold and yeast and high contamination count to mesophilic bacteria. Only one case (0.3%) of samples had contamination of combined with high counts of mesophilic bacteria and identified *Enterococcus*. Besides, one sample (0.3%) associated with high count of contamination of mesophilic bacteria, mold, yeast and *Pseudomonas* was observed. Of rose water samples, 72 (37.7%) of the total count of mesophilic bacteria were uncountable and 85 (44.5%) of their total bacteria counts were less than 100. Total bacterial counts were between 10^2 to 6×10^3 cfu/ml for 34 (17.8%) of samples, which had an average of $1.656\times10^3\pm2.031\times10^3$ cfu/ml. The comparative distribution of contamination of herbal water and rose water samples based on microbial tests are presented in Table 1.

Based on the obtained results, there was no significant difference between usability and non-usability of distributed herbal waters and rose waters ($p>0.05$). The results gained from the microbial tests presented as acceptability and unacceptability of herbal water and rosewater samples based on the national standard of Iran are shown in Table 2.

Also, there was significant difference between usability and non-usability conventionally herbal water samples and the industrially herbal water samples ($p<0.05$). 24.68% conventionally herbal waters and 52.52% industrially herbal water samples were usable.

The comparative distribution of herbal water samples which were consumable based on traditional and industrial process are presented in Table 3. The maximum permitted number of bacteria, based on the national standards of Iran, can be seen in Table 4.

Table 1. Comparative distribution of contamination of herbal water and rose water samples based on Microbiological tests

	Microbiological tests							Total
	Total bacteria count (cfu/ml)*	*Coliforms*	*Enterococcus*	*Pseudomonas*	*Sulfite-reducing Clostridia*	Mold	Yeast	
Kind of herbal products								
Herbal waters	$1.153\times10^3\pm1.738\times10^3$	0.35% (1)	0.7% (2)	0.35% (1)	0% (0)	10.5% (27)	12.9% (33)	100% (256)
Rose waters	$1.656\times10^3\pm2.031\times10^3$	0% (0)	0.53% (1)	0% (0)	0% (0)	5.76% (11)	9.43% (18)	100% (191)

*Average ± SD for Total bacteria count

Table 2. Comparative distribution of consumability of herbal water and rose water samples based on Iranian national standards

Microbiological tests	Usable		Unusable	
	Herbal waters	Rose waters	Herbal waters	Rose waters
Total bacterial count	48.82%	44.5%	51.18%	55.5%
Mold	89.5%	94.24%	10.5%	5.76%
Yeast	87.1%	90.57%	12.9%	9.43%
Coliforms	99.7%	100%	0.3%	0%
Enterococcus	99.3%	99.47%	0.7%	0.53%
Pseudomonas	99.7%	100%	0.3%	0%
Sulfite-reducing Clostridia	100%	100%	0%	0%

Table 3. Comparative distribution of consumability of herbal water samples based on traditional and industrial process

Kind of process	Usable	Unusable	Total number
Traditional	19 (24.68%)	58 (75.32%)	77
Industrial	94 (52.52%)	85 (47.48%)	179

Table 4. microbiological properties of herbal waters and rose waters based on Iranian national standards

Maximum limit in 100 cc	Microbiological properties						
	Total bacterial count	Mold	Yeast	*Coliforms*	*Enterococcus*	*pseudomonas*	*sulfite-reducing Clostridia*
Herbal waters	200 cfu/ml	0	0	0	0	0	0
Rose waters	100 cfu/ml	0	0	0	0	0	0

Discussion

Based on the obtained results, 113 cases of herbal water (44.15%) and 83 rosewater (43.45%) samples were non-usable based on the national standards of Iran. That was due to the excessive existence of aerobic mesophilic bacteria, mold and yeast, *coliforms* detection, identification of *Pseudomonas* and *Enterococcus* in them. However, in none of the samples, *E. coli* and *sulfite reducing Clostridia* was observed. Given that none of the health indicator microorganisms, especially fecal contamination (18) was isolated from the samples, it might be indicated that compliance of employees with the terms of individual hygiene and cross contamination are during production and post-production of herbal waters and rose waters. However, the presence of one case of *Pseudomonas* and one case of *Enterococcus* in herbal waters caused it to be non-usable. As mentioned above, the samples was diagnosed as non-usable according to the total count of mesophilic bacteria by pour plate method and mold and yeast count based on membrane filtration based on the national standard of Iran. In a way that, out of 246 cases (55.03 %) of the samples, the total amount of bacterial count exceeded the standard level and 37 (8.27%) and 51 (11.4%) cases of the samples have been out of standard limit according to the mold and yeast count.

These infections can be due to the lack of pasteurization, the use of contaminated glass and plastic bottles and lack of proper cap-packaging of herbal waters and rose waters (19).

According to the National Public Health Service (NHS), pour plate method of counting bacteria alone cannot be used to show the dangers threatening food safety, such as water. The method is more appropriate to evaluate the healthiness of groundwater resources and to determine the efficiency of water refining processes such as coagulation, filtration and disinfection and can indicate the health and cleanliness of the water distribution system (7,19).

The present study was consistent with the study of Soltan Dallal *et al.* (7). In this study, the application of membrane filtration technique to detect *Coliforms* and *E. coli*, along with other microorganisms such as *pseudomonas aeruginosa, Enterococcus, sulfite-reducing Clostridia* was diagnosed as appropriate.

In a way that, for example, most probable number method (MPN) can increase application of medium membrane filtration technique to detect mentioned microorganisms in liquid foods such as herbal waters and rose waters for the detection of *Coliforms* and *E.coli* due to the time-consumability, hard labor of workforce needs to a large area for culture (7). So far, a few studies have been done for the microbial quality of herbal waters and rose waters. In a study in 2005, microbial quality of rose waters distilled by conventional and industrial methods by production manufactory in Kashan showed that 63.97% of all the samples were non-usable from Iranian national standards point of view. In a way that, all conventionally distilled samples were non-usable and 35.41% of the industrial rosewater samples were usable. It is also suggested that the traditional manufactory of rosewaters be converted to industrial plants (19). In the present study, the 43.45% of distributed rosewaters were usable. Also, 75.32% conventionally herbal waters and 47.48% industrially herbal water samples were non-usable. In a study by Khodadi *et al.*, 80% of traditional herbal waters in Birjand were microbial contaminated, but this contamination was observed in 60% of industrially herbal waters. In addition, in another study by Fazl Ara *et al.*, in 14.81% of the traditionally herbal waters *Enterococcus* spp. were identified by impedance method (1,20).

The results of this study indicate the microbial contamination of both herbal waters and rose waters but in none of the samples, microbial indicators of fecal contamination was detected.

Despite the antibacterial properties of various medicinal plants and herbal waters prepared from them, one of the reason for bacterial activity observed in the understudy herbal waters may be to due lack of hygiene practices during their collection and distillation. It may also be due to their antibacterial effects reduction as they are diluted.

Conclusion

Based on the results of this study, it is suggested that manufacturers of herbal waters pay attention to the hygiene observance, Good Hygiene Practice (GHP), the use of pasteurization temperatures, observance of condition of the manufactory and Good Manufacturing Practices

(GMP) and appropriate packaging in the manufacturing processes to reduce secondary contamination and enhance the quality of the final product.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of interests

The authors declare no conflict of interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the author.

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