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Evaluating the microbial contamination of some Iranian dried medicinal plants and distillates

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ABSTRACT

Background and aims: In Iran herbal water and dried herb are as traditional medicinal and are consumed widely. Therefore, microbial evaluation of these products in term of public health is important. The aim of the present study was to study the contamination of some dried medical plants and distillates in the groceries of Shahrekord city.

Methods: In this descriptive study, 35 samples of herbal waters and 35 samples of 7 species (lavender, lemon balm, valerian, savory, borage, mint and thyme) dried medicinal plant (total samples=70) distributed in Shahrekord during spring to summer of 2012 were purchased and transferred to laboratory. Then, microbial tests such as total aerobic bacterial count mold and yeast count, total coliforms, and detection were evaluated based on national standard of Iran.

Results: Contamination to mesophilic bacteria and yeast was observed in the 100% of distilled samples, based on the national standard of Iran. Additionally, none of the medicinal dried plant was contaminated according to the national standard of Iran.

Conclusion: According to contamination of all distilled, It is proposed that producers of herbal distillates consider the hygiene conditions, using correct and suitable pasteurization, considering the structural condition of workshop and also appropriate packaging in order to reduce the secondary contamination and increase the quality of the finished product.

Keywords: Medicinal dried plants, Distillates, Microbial load, Coliforms, *Escherichia coli*.

Original article

INTRODUCTION

Medicinal Plants are of great value and importance in providing the health of communities in both treatment and prevention of disease. The use of traditional

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medicine, especially the plant originated medicines, has increased in the world widely since 1990 because of being cost effective, availability and their role in providing the basic necessities of healthcare in many developing countries in Africa and Asia.[1] Consumers of the traditional medicines in South Africa and Asia are estimated 200,000, in consultation with 60% of the consumers in South Africa and Asia, it was concluded that the traditional medicines are as their basic source of health and hygiene.[2] In Iran, herbal water and dried herb are as traditional medicinal and are consumed widely. Therefore, microbial evaluation of these products in term of public health is important.[3]

To date, much of the herbal research in the corresponding field is focused on the isolation and the evaluation of active and toxic compounds in the herbal medicine to validate and strengthen the security and efficiency. However, the researches on the assessment of toxic substances and other contaminants which have detrimental effects on plants have been carried out.[4] The contamination of micro-organisms may lead to the disease.[5] In previous research on herbal medicines, pathogenic microorganisms, and several fungal species such as Aspergillus, Penicillium and Fusarium resistant to methicillin and vancomycin have been found.[6-8] So, the safety of infected plants in the external markets should be paid attention in which the conditions for microbial proliferation are provided.

Most previous studies have been carried out on the infection of medicinal plants by microbes and fungal species, that focus on the bacterial infections and microbial loads. Collecting and handling the medicinal plants are not always in sanitary conditions that can lead to a lots of microbes and thus cause some damages.[9,10] Most herbs are dried in exposed to air that may develop the infections caused by the bacterial and fungal in the air and soil. The microbial infections of plants limit the use in food products, pharmaceutical and cosmetics industries. The hygienic quality of medicinal plants, as well as the use of antiseptic methods, is an important step towards the consumer health and treatment effectiveness. Microorganisms important in the public health(as pathogens) including *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Clostridium perfringens (C. perfringens), Bacillu scereus* and Salmonella, can exist in spiceplants. The final microbial status of plants is determined by the natural numbers of microorganisms in the plants, and by operations such as harvesting, drying, transporting and packing process.[11-13] The study of Khodadadi in Birjand City found that 80% of traditional distillates were positive for coliforms and non coliforms.[14] Also, Mazroii and colleagues reported herbal extracts distributed in Kashan were contaminated with mesophilic aerobic bacteria and yeast (51/52%, 11/6% respectively).[3]

Considering the importance of this issue and expand the use of herbal medicine and medicinal plants distilled in our country, the study of the possibility of microbial and fungal becomes necessary.

METHODS

A total of 35 medicinal dried plants and 35 distillates samples from 7 plants (lavender, lemon balm, valerian, savory, borage, mint and thyme) were collected randomly from 5 different groceries in Shahrekord city, in the spring and summer 2012. Then, all of samples were transported to the microbiology laboratory of the Islamic Azad University of Shahrekord to investigate the microbial status to load and were tested as soon as possible.

The results were analyzed by SPSS and Chi-square and Fisher exact models with a confidence level of 95%. The test was considered significant correlation between the two data.

Microbial load of medicinal dried plant samples, the number of total aerobic bacterial count, coliforms, *E. coli, S.aureus,* molds and yeasts were tested. The basic dilution of 10^{-1} was prepared firstly from the samples.[14] For this purpose, 25 grams of the solid samples were added into 225 ml peptone water broth, and the bacterial counting was performed in each case as follows: To count the total number of bacteria, according to ISO 4833 reference method, 5 tubes containing 9 ml of water peptone broth were selected and by transferring1 ml of the basic dilution into the first tube, and preparing the serial dilutions, the dilutions of 10^{-1} to 10^{-6} were prepared from samples.[16-18] 1 ml of the dilutions (10^{-1} to 10^{-6}) were added into two empty plates, followed by adding melt plate count agar medium (45°C), and incubation at 30°C for 72 hours. In the following, the two plates related to the dilutions containing approximately 30 to 300 colonies were chosen and their numbers were multiplied by the inversely related dilutions. The number of bacteria in 1 ml of the sample was reported in terms of CFU.[19-21] The counting of coliform bacteria was performed according to ISO 21528-2 and ISO-4832 reference methods similar to previous steps, and melt MacConkey agar medium (Merck ink, Darmstadt, Germany) was used.[22,23] It was incubated at 37°C for 48 h. to count the mold and yeasts, the medium of Extract Glucose Chloramphenicol Agar (YGCA; Merck Ink,Darmstadt, Germany) and the steps were performed as above.then incubated at 25 °C for 3 for 5 days.

For microbial load assay for distillates samples, 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.[24]

To count mold and yeast, 100 ml of sample was 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.[25]

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 Mac Conkey agar medium (Merck ink, Darmstadt, Germany) by means of a sterile forceps. It was incubated at 37°C for 24 to 48h 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).[26]

Finally, after counting the bacteria colonies, if present, to analyze the data, and compare mean, fisher test method and SPSS Ver.20 Statistical software were applied.

Table 1: List of the medicinal plants that their microbial load was investigated

Plant name	Family	Scientific name	TheUsed section
Mint	*Lamiaceae*	*Mentha spicata*	Leaf
Shirazi Thyme	*Lamiaceae*	*Zataria multiflora*	Leaf
Savory	*Lamiaceae*	*Satureja hortensis*	Leaf
Valerian	*Valerianaceae*	*Valeriana wallichii*	Rhizome
Lemon balm	*Lamiaceae*	*Melissa officinalis*	Leaf
Borage	*Boraginaceae*	*Echium amoenum*	Leaf and flower
Lavender	*Labiatea*	*Lavandula stoechas* L	Leaf

RESULTS

The result of microbial evaluation of dried plant and its distillates distributed in Shahrekord were shown in Table (2 and 3).

Table2: Evaluation of themicrobial loadof various types of dried aromatic vegetables

Sample Type	No. of samples	The total number ofbacteria	Coliforms	*Esherishia.coli*	Mold
Lavender	5	4.78×10^{2}	7.6×10^{1}	-	-
Lemon balm	5	1.41×10^{3}	6.8×10^{1}	-	-
Valerian	5	5.95×10^{2}	7.8×10^{1}	-	-
Savory	5	9.86×10^{2}	2.8×10^{1}	-	-
Borage	5	2.27×10^{3}	9.72×10^{1}	-	-
Mint	5	1.81×10^{3}	7.24×10^{1}	-	-
Thyme	5	3.8×10^{3}	0.92×10^{1}	-	-

Table 3: Evaluation of themicrobial load of various types of distillates

Sample Type	No. of samples	The total number of bacteria	Coliforms	*Eshirishia.coli*	Mold	Yeast
Lavender	5	1.28×10^{5}	Negative	Negative	Negative	1.418×10^{3}
Lemon balm	5	6×10^{4}	Negative	Negative	Negative	1.78×10^{2}
Valerian	5	4.88×10^{6}	Negative	Negative	Negative	3.025×10^{3}
Savory	5	4.328×10^{6}	Negative	Negative	Negative	1.236×10^{3}
Borage	5	7.1246×10^{6}	Negative	Negative	Negative	2.02×10^{2}
Mint	5	4.103×10^{6}	Negative	Negative	Negative	1.53×10^{3}
Thyme	5	4.124×10^{6}	Negative	Negative	Negative	4.84×10^{2}

Based on the results gained from the total sample of dried plant, despite observation contamination count to mesophilic bacteria, according to national standards of Iran, all samples were usable (Table 4).[27]

Table 4: The microbial standard of microbiolog of dehydrated vegetables specifications

Properties	Admissible limit (gr)
Total count	10^{5}
Coliforms	10^{3}
E. coli	Negative
Mold	10^{3}

21 cases (60%) of distillates showed contamination to yeast and high contamination count to mesophilic bacteria ($P<0.05$). Total bacterial counts and yeast were between 10^{2} to 3.2×10^{7} cfu/ml and negative to 7.5×10^{3} respectivly. 40% of sample were usable based on the national standard of Iran (Table 5).[28]

Table5: The microbial standard of herbaceous distillates specifications

Properties	Admissible limit in 100 ml
Total count	200 cfu/ml
Coliforms	Negative
E. coli	Negative
Mold	Negative
Yeast	Negative

DISCUSSION

Based on the obtained results, dry medicinal herbs were consumable according to Iran National Standard. While 60% of distillate samples were contaminated with aerial bacteria neutrophil and yeast, theywere not consumable. However, contamination with Coliform and *E.coli* was not seen in any distillate sample, showing the individual hygiene of personnel and non-fecal contamination.

Chen et al found in a research on medicinal plants and spices that ginger is free of bacteria Sospedra et al observed in a study on the microbial contamination of medicinal plants and spices no bacterial contamination in thyme, basil, cloves, ginger and parsley has seen.[29,30]

Microorganisms existing in dry herb are among the native microorganisms of soil and plants. Dust, inset, wastes of birds and rodents and consumed water in the process are some other contamination resources of this product.

Microbiological quality of dry herbs is mostly depended on the culture region, transportation condition and storage of these products. It is not possible to grow the pathogen microorganisms in this product due to its water activity. If drying process and storage condition of dry herb are suitable, microorganisms can not grow in it.[27]

Contamination in the distillate can be resulted from lack of pasteurization, inappropriate pasteurization, using the contaminated glass or plastic bottles and lack of suitable sealing.[31]

There are limited studies about microbial quality of herbal distillates. In a study on the microbial quality of industrial and traditional produced rosewater in Kashan, Iran, 63.97% samples were reported as non-consumable according to Iran National Standard, so that all traditional-produced samples were contaminated and non-consumable.[31] In a research done by Khodadadi et al on the supplied distillates in Birjand, it was observeed 80% contamination in the traditional distillates and 60% in the industrial distillates.[14] Studies done by Mazrui et al on 132 samples of herbal distillates supplied in Kashan, Iran showed 51.52% and 11.6% contamination to aerial bacterial neutrophil and yeast, respectively, but contamination with California and *E.coli* was not seen.[3] Researchers studying the traditional-produced distillates in Owino market reported that all samples have neutrophil aerial bacterial contamination.[32]

These studies are all consistent with the results of the recent study showing that distillate samples have contaminated with aerial bacteria mesophil and yeast.

In general, in addition to the previous reasons for contamination of distillates, the following cases can be mentioned. Disrespecting the hygiene hints during collecting and providing the distillates and also dilution of these distillates result in contamination in the distillates.[14] These contaminations can have undesirable effects on the health of consumer.[32]

CONCLUSION

Then, based on the obtained results, it is proposed that producers of herbal distillates consider the hygiene conditions, using correct and suitable pasteurization, considering the structural condition of workshop and also packaging appropriate in order to reduce the secondary contamination and increase the quality of the finished product.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

1. Kneifel W, Czech E, Kopp B. Microbial contamination of medicinal plants: A review. Planta Med. 2002; 68(01): 5-15.
2. VanWyk B, Van Oudtshoorn B, Gericke N. Medicinal plants of South Africa. 2nd ed. South Africa: Briza Pub; 2009: 95-100.
3. Arani NM, Chaleshtori RS, Rafieian Kopaei M. Microbial quality of some medicinal herbal products in Kashan, Iran. J Herb Med Pharmacol. 2014; 3(2): 113-7.
4. World Health Organization (WHO). Background of WHO Congress on Traditional Medicine. Available from: http://www.who.int/medicines/areas/traditional/congress/congress_background_info/en/ (Accessed 21/02/2014).
5. Govender S, Du Plessis-Stoman D, Downing T, Van de Venter M. Traditional herbal medicines: Microbial contamination, consumer safety and the need for standards: Research letter. South Afr J Sci. 2006; 102(5-6): 253-5.
6. Street R, Stirk W, Van Staden J. South African traditional medicinal plant trade: Challenges in regulating quality, safety and efficacy. J Ethnopharmacol. 2008; 119(3): 705-10.
7. Yahaghi E, Imani Fooladi AA, Amin M, Mirnejad R, Nezamzade R, Amani J. Detection of class I integrons in *Staphyloacoccus aurous* isolated from clinical samples. Iran Red Crescent Med J. 2014; 16(11): e16234.
8. Aziz NH, Youssef YA, El-Fouly MZ, Moussa LA. Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. Bot Bul Acad Sin. 1998; 39: 279-85.
9. Stickel F, Droz S, Patsenker E, Bogli-Stuber K, Aebi B, Leib SL. Severe hepatotoxicity following ingestion of Herbalife nutritional supplements contaminated with *Bacillus subtilis*. J Hepatol. 2009; 50(1): 111-7.
10. McKee L. Microbial contamination of spices and herbs: A review. Lwt-Food Sci Technol. 1995; 28(1): 1-11.
11. Little CL, Omotoye R, Mitchell RT. The microbiological quality of ready-to-eat foods with added spices. Int J Environ Health Res. 2003; 13(1): 31-42.
12. Ernst E. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends Pharmacol Sci. 2002; 23(3): 136-9.
13. WHO. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO; 2007.
14. Khodadadi M, Motamed Rezaei O, Jahani M, Dorri H. The survey on Bacterial and fungi contamination of herbaceous distillates that distributed in Birjand city. J Birjand Univ Med Sci. 2012; 19(1): 51-8.
15. Soriani RR, Satomi LC, Terezinha de Jesus AP. Effects of ionizing radiation in ginkgo and guarana. Radiat Phys Chem. 2005; 73(4): 239-42.
16. Iten F, Reichling J, Saller R. Adverse effects and interactions of phytotherapeutic drugs, in German. Ther Umsch. 2002; 59: 91-283.
17. Nemtanu MR, Brasoveanu M, Minea R, Grecu MN, Albulescu M, Mitru E. Microbiological decontamination of Spirulina platensis and green coffee using accelerated electron beams. Rom J Biophys. 2006; 16(2): 141-8.
18. Gould GW. Methods for preservation and extension of shelf life. Int J Food Microbiol. 1996; 33(1): 51-64.
19. Miwa N, Masuda T, Terai K, Kawamura A, Otani K, Miyamoto H. Bacteriological investigation of an outbreak of Clostridium

perfringens food poisoning caused by Japanese food without animal protein. Int J Food Microbiol. 1999; 49(1-2): 103-6.
20. Kunene NF, Hastings JW, Von Holy A. Bacterial populations associated with a sorghum-based fermented weaning cereal. Int J Food Microbiol. 1999; 49(1-2): 75-83.
21. Tournas VH, Katsoudas E, Miracco EJ. Moulds, yeasts and aerobic plate counts in ginseng supplements. Int J Food Microbiol. 2006; 108(2): 178-81.
22. Banerjee M, Sarkar PK. Microbiological quality of some retail spices in India. Food Res Int. 2003; 36(5): 469-74.
23. Martins HM, Martins ML, Dias MI, Bernardo F. Evaluation of microbiological quality of medicinal plants used in natural infusions. Int J Food Microbiol. 2001; 68(1-2): 149-53.
24. Institute of Standards and Industrial Research of Iran. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of microorganisms: Colony count technique at 30°C. ISIRI Number. 5272, 2007. Available from: https://en. wikipedia.org/wiki/Institute_of_Standards_a nd_Industrial_Research_of_Iran.
25. Institute of standards and industrial research of Iran. Water quality enumeration of microorganisms in water by culture: Guideline. ISIRI Number 4207, 2007. Available from: https://en.wikipedia.org/ wiki/Institute_of_Standards_and_Industrial_ Research_of_Iran.
26. Institute of standards and industrial research of Iran. Water quality: Detection and enumeration of *Escherichia coli* and coliform bacteria. ISIRI; Number 7725-1, 2004. Available from: https://en.wikipedia. org/wiki/Institute_of_Standards_and_Industr ial_Research_of_Iran.
27. Institute of standards and industrial research of Iran. Microbiolog of dehydrated vegetables: Specifications. ISIRI Number 5939, 2008. Available from: https://en. wikipedia.org/wiki/Institute_of_Standards_a nd_Industrial_Research_of_Iran.
28. Institute of standards and industrial research of Iran. Microbiology of herbaceous distillates: Specifications. ISIRI Number 3545, 2007. Available from: https://en.wikipedia.org/wiki/Institute_of_St andards_and_Industrial_Research_of_Iran.
29. Sospedra I, Soriano JM, Manes J. Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. Plant Foods Hum Nutr. 2010; 65(4): 364-8.
30. Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Foods Hum Nutr. 2008; 63(1): 15-20.
31. Abedi Mohtasab TP, Hydarzadeh Arani H, Dolati MA, Hossieni SA. Microbial quality of traditional vs industrial golab in Kashan. Proceeding of the 9th IRanian Nutrition Congress; 2006.
32. Rehema G. An assessment of bacterial contamination of locally prepared herbal/traditional oral liquid medicines sold in owino market. 13 th Congress on Public Health. Addis Ababa, Ethiopia; 2012.

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