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A SURVEY ON MICROBIAL QUALITY OF HERBAL DISTILLATES IN ISFAHAN, CENTRAL OF IRAN

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ABSTRACT: Studies have shown that over than 80% of the people in the world use traditional drugs to treat their diseases. Regarding to high consumption and pharmaceutical use of herbal extracts, microbial qualities of eight herbal extracts including lavender, *Fumaria parviflora*, pussy, cumin, mint, camel's thorn, rose water and *Melissa officinalis* were investigated in this study. 64 samples of eight type herbal extracts retailed in Isfahan markets were purchased randomly and their total bacterial count, including contamination with coliforms, sulphite reducing *Clostridium, Entrococci*, *Pseudomonas aeroginosa*, molds and yeasts were evaluated according to Iranian national standard protocols. 37% of total samples were unacceptable and not consumable compared with the protocol of the national standard of Iran. Forty numbers of samples (63% of total samples) were acceptable and consumable. For improving 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 are necessary.

Keywords: Herbal extracts, Microbial contamination, Isfahan, Iran

INTRODUCTION:

Plants and trees are used from the beginning of man creation to provide food, clothing, housing, transportation, fuel, tools and animal grazing (Sewell and Rafieian-Kopaei, 2014). Medicinal plants and their natural products can also be used to prevent or treat a variety of diseases (Bahmani *et a*l., 2014; Delfan *et al*., 2014; Rabiei *et al*., 2014; Rafieian-Kopaei *et al*., 2014).

Recent trends in the world of medicine have been associated with public interest in nations to use natural methods for prevention and treatment of diseases (Sarrafchi and Rafieian-Kopaei, 2014; Bahmani *et al*., 2014; Asadi-Samani *et al*., 2014) .

Studies have shown that more than 80 percent of the world's people use traditional medicine for their diseases treatments (Karamati *et al*., 2014; Delfan et al., 2014; Bahmani and Rafieian-Kopaei, 2014; Amirmohammadi *et al*., 2014). Given the positive effects of medicinal herbs and their fewer side effects, cheapness, effectiveness, acceptability and availability to people, laboratory and clinical researches increased and is ongoing to understanding their health effects (Asgary *et al*., 2013; Bahmani *et al*., 2014; Saki *et al*., 2014; Khosravi-Boroujeni *et al*., 2012; Bahmani *et al*., 2014).

Use of Ethnobotany and traditional medicine knowledge and traditional supplements has increased the discovery factors of natural medicines up to forty percent, while the likelihood of drug discovery in random studies without traditional support is only about one percent (Bahmani *et al*., 2014; Ghasemipirbalouti *et al*., Bahmani *et al*., 2013; Bahmani *et al*., 2015; Eftekhari *et al*., 2012). Use of herbal drugs in different nations is being spread increasingly, and their known compounds are increasingly introduced as new drugs for treatment of various diseases (Asadbeigi *et al*., 2014; Khosravi-Boroujeni *et al*., 2013; Sadeghi *et al*., 2014).

Medicinal plants derived compounds play a crucial role in their health care systems, so that they are allocated about 20 percent of health care services. Medicinal plants can be used to make semi-synthetic compounds or traditional forms of drug dosage forms that are used in traditional or semi-synthetic include herbal extracts, essential oils, etc (Bahmani *et al*., 2015; Delfan *et al*., 2015; Bahmani *et al*., 2015; Gholami-Ahangaran *et al*., 2012; Bahmani *et al*., 2013; Bahmani *et al*., 2015).

One of the most important pharmaceutical forms of medicinal herbs that are effective and widely used is herbal extracts. Herbal extracts are used for prevention and treatment of diseases or as a food flavoring and aroma agent, in Iranian traditional medicine (Delfan *et al*., 2014; Asgary *et al*., 2013; Sarrafzadegan *et al*., 2013).

According to Iranian National Standards Institute, herbal extracts are aromatic distilled waters and saturated solutions of volatile plant oils or other aromatic substances in water which are obtained from fresh or dried plant organs (ISISI, 2012).

Herbal extracts affect in different ways, including: Olfactory receptor neurons stimulation, effects on emotional and sensual thoughts, behavior changes, stimulation of hormone release, increase in body metabolism, changes in levels of sex and stress hormones and immune system ability (ISISI, 2012).

Natural remedies such as herbal extracts are widely consumed as juice, drinks, snack foods or drugs. If manufacturing process of these compounds is not

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correct, various types of contamination may occur in these natural products.

Medicinal plants, foods, spices and so on may become contaminated with various environmental pollutants (industrial or non-industrial contamination) such as: hexachlorocyclohexan, hexachloride, pentachloro nitrobenzene, dichloro diphenyl trichloro ethane, methanol, heavy metals such as arsenic, cadmium, mercury and lead (Sahoo *et al.*, 2010; Teimouri *et al.*, 2014).

Reports show that in developing countries herbal extracts are recognized as pharmaceutical medicine and are used as Complementary Medicine, but with no hygienic conditions without adequate supervision are released to market. Hence, high microbial contamination may occur in these products (Ali *et al*., 2005; Sharafati *et al*., 2011). Therefore, attention to health implications of herbal extracts is important . The aim of this study was evaluation of the microbial quality of eight herbal extracts including lavender, *Fumaria parviflora*, pussy, cumin, mint, camel's thorn, rose water and *Melissa officinalis*, retailed in Isfahan city of Iran.

MATERIALS AND METHODS:

Sampling

64 samples of eight types of herbal extracts were taken from Isfahan cities markets in Iran. 8 samples of each type of herbal extracts of lavender, *Fumaria parviflora*, pussy, cumin, mint, camel's thorn, rose water and *Melissa officinalis* were taken for evaluating microbial quality.

Microorganisms total count

Total count of microorganisms was done using Iranian national standard No. 5272. Acceptable limit for microorganisms in this method was 10^2 CFU/gr. Serial dilutions of samples were prepared and dilutions of 0.1 and 0.01 of samples were plated on PCA culture medium and incubated for 3-2 days at 30 °C. The colonies were counted regarding to dilutions (INS, 2012; Bagheri *et al*., 2013).

Enumeration of coliforms

Coliform counts were performed using Iranian national standard No 7725-1. Acceptable limit of microorganisms was considered to be negative (zero). TTC Lactose agar medium was used for bacteriological counting of filtered samples and incubated for 1-2 days at 37 ° C. In case of yellow halo around the colored colonies, oxidase test was done and negative reaction indicated coliforms (INS, 2013).

Enumeration of pseudomonas aeroginosa

Iranian national standard method No. 8869 was used to enumerate *Pseudomonas aeroginosa*. Acceptable limit of microorganisms was considered to be negative (zero). CT agar (Cetrimide agar) medium was used for bacteriological counting of filtered samples and incubated for 1-2 days at 42 °C. In case of suspect fluorescence colonies confirmatory test was included and xidase and catalase tests were done. Both tests were positive for Pseudomonas aeruginosa (INS, 2013).

Enumeration of Enterococci

Enterococcus bacteria enumeration was performed using Iranian national standard No 7724-2. Acceptable limit of microorganisms was considered to be negative (zero). 250 ml herbal extracts were filtered under aseptic conditions. The corresponding filters were placed on kf agar medium (prepared at the plate), and incubated at 37 °C for 1-2 days. In case of cherry colored colonies, confirmatory tests were performed on the bile esculin agar medium and incubated at 44 °C, and after 2 hours up to 1 day, maroon colored colonies were *enterococci* (INS, 2013).

Enumeration of sulphite reducing Clostridium

Enumeration of sulphite reducing *Clostridium* was done using Iranian national standard No. 5353. Acceptable limit of microorganisms was considered to be negative (zero). At first provided filters of samples were put into sterile plates and then iron sulfite agar (ISA) medium was plated and incubated at 37 °C in anaerobic jars for 48 hours. Black colonies indicated *clostridium* bacteria (INS, 2013).

Enumeration of mold and yeasts

Mold enumeration was done using Iranian national standard No. 4207. Acceptable limit of microorganisms was considered to be negative (zero). At first provided filters of filtered samples were put into sterile plates. DRBC agar (Dichloran Rose-Bengal Chloramphenicol Agar) medium was added on filters and incubated for 3-5 days at 25 °C. The plates were checked for the presence of molds and yeasts (INS, 2013).

RESULTS:

Obtained results of herbal extract samples supplied in Isfahan city are shown in table 1. For each type of 8 herbal extract two items including bacterial and fungal contaminations were checked and the results with details of each type are outlined. Results showed that 37% of total samples were unacceptable in comparing with national standard of Iran. 18.75 % of samples were contaminated with coliforms higher than standard level. All samples were negative for sulphite reducing Clostridiums, Entrococci and *P. aeruginosa*. 100% of samples were negative for presence of molds but 27% of samples were positive for yeasts.

The results showed that a quarter of *Fumaria parviflora*, lavender, pussy, cumin of samples were within an unauthorized range. Also, 62.5% of samples mint, Camel's thorn and Rosewater were found in an unauthorized range. 12.5% of the sample *Melissa officinalis* was reported in an unauthorized range.

Unauthorized reasons of samples are specified in Table 1.

Table 1.
Results of herbal extract samples supplied in Isfahan city

Original plant of Herbal Extract	Total sample (64)	No. of rejected samples	Reason of Rejection
Lavandula	8	2	High total count, Coliforms presence
Fumaria parviflora	8	2	High total count
Pussy	8	2	High total count, Yeasts presence
Cumin	8	2	High total count, Yeasts presence
Mint	8	5	High total count, Coliforms presence
Camel's thorn	8	5	High total count, Coliforms and Yeasts presence
Rosewater	8	5	High total count, Coliforms and Yeasts presence
Melissa officinalis	8	1	High total count, Yeasts presence

DISCUSSION:

Medicinal plants have been used for a long time by Iranians and Iranian traditional medicine is considered as one of the most precious gifts of God. Thousands years experiences of science of medicinal plants can help us to resolve some health problems and diseases of society (Sewell and Rafieian-Kopaei, 2014).

Enjoying the directives contained in it, like before, physicians can help resolving many health and therapeutic problems. Obtained results showed that microbial quality of 37% of tested herbal extracts were unacceptable.

Herbal extracts are medical products that Iranians have developed them and widely used, but few studies have been conducted on their hygienic and microbial quality in Iran. Since essential oils and herbal extracts from the root bark herbs or petals in water are prepared traditionally, so the likelihood of microbial contaminations in these beverages would be immense. The pollution might be by lack of hygiene standards in the manufacturing process that can be secondary contamination by fecal bacteria (coliforms), molds or yeasts.

Contaminations may also result from pre-harvest and post-harvest factors, improper handling practices, storage and packaging conditions of herbal distillates. Acquired herbal extracts from the distillation process are sterile and free from any contamination. Possibility of contaminations might be through devices, individuals, packaging and unhygienic conditions in both industrial and traditional manufacturing. For this reasons traditional herbal extracts are not free from contaminations.

There would be fewer concerns about contamination, if herbal distillations are prepared in industrial manufactures or in small workshops with providing good producing health conditions, clean containers for packaging and following sanitation by producers.

Reports indicated that fungal species isolated from the herbal medicines mainly were *Aspergillus* and *Penicillium* molds, and more isolated fungi capable of producing mycotoxins and particularly aflatoxins (Sahoo *et al*., 2010; Carlile *et al*., 2001).

In a study on the total count of aerobic bacteria, coliforms, molds and yeasts in industrial and traditional product of rose waters in Kashan city of Iran , 63.97 % of traditional products had microbial contaminations and were not consumable. However, 64.59% of industrial products were consumable (Abedi Mohtasab, 2006; www.isiri.ir). Khodadadi and coworkers study showed the presence of *penicillium* mold in a sample of *Teucrium polium* (TP) and forty plants traditional herbal extracts, and also *Aspergillus* and *penicillium* fungi from traditional sweat chicory in Birjand of Iran, 80% of the traditional sweat chicory and *Teucrium polium* herbal extract samples had microbial contamination with coliform and non-coliform bacteria. 25% of traditional chicory, forty plants and *Teucrium polium* herbal extracts were contaminated with fecal coliforms. 60% of industrial product samples of sweat chicory had contaminations with fecal coliform (Khodadadi *et al*., 2012).

CONCLUSION:

Due to the therapeutic effects of herbal extracts, their high production and usage, more and more attention in relation with the production of safe beverages is recommended .

The use of contaminated herbal extracts can reduce therapeutic efficacy and cause adverse effects. For improving 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 are necessary.

For the hygiene products GMP and HACCP all the factories producing these products should be adhered. The staff have a health card and healthy water and water of free from contamination used in the production process.

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