

The Survey of Microbial Quality of the Dry Sample, Extract and Brewing of some Medicinal Plants

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Abstract

Medicinal plants may be exposed to a wide range of microbial contamination during pre- and post-harvest stages and they can present high microbial counts. In this study, the microbial quality of 44 samples of dry herbs namely: mint (*Mentha* spp.), lemon balm (*Melissa officinalis*), summer savory (*Satureja hortensis*), zataria (*Zataria multiflora*), Indian valerian (*Valeriana wallichii*), their brewing and extracts were analyzed. Total count using plate count agar medium (PCA), coliform count by Violet Red Bile Agar (VRBL), Enterobacteriaceae by Violet Red Bile Glucose (VRBG) were evaluated. Medium Baird-Parker agar (BP) medium and Tryptone Bile X-Gluc (TBX) medium were used for the isolation and enumeration of *Staphylococcus aureus* and *E. coli* spp. respectively. Furthermore, Xylose Lysine Deoxycholate agar medium (XLD) and Bismuth Sulfite Agar medium (BSA) were used for detection of *Salmonella* spp. Fungal and mold contamination was assessed using yeast extract glucose chloramphenicol agar. The results showed that the contamination of the samples with total count (100%) and Enterobacteriaceae (85%), total coliform (83%), mold and yeast (98%) and *E. coli* spp. (2.27) were detected, including in the study samples the absence of pathogenic bacteria like *Staphylococcus aureus*, *Salmonella* spp. Moreover, the extract had a lower microbial load in comparison to dry herb samples. Also, the lowest and the highest of contamination rates were observed for Indian valerian and zataria, respectively. According to the results, there is a need to control the environmental conditions and improve hygiene in the production process; even more, it is recommended to choose a suitable decontamination method for disinfection during packing medicinal plants and during post-packing manipulation and transport.

Keywords: contamination, *Escherichia coli*, microbial load, *Salmonella* spp., *Staphylococcus aureus*

Introduction

Medicinal plants are important considering human health, being a valuable tool for disease prevention and treatment. General trends use drugs and therapies with herbal and natural products and are increasing their application during recent years, due to proved negative effects of chemical drugs and environmental pollution. But the collection and handling of medicinal plants are not usually done in sanitary conditions and differences in cultivation conditions can also increase pollution, influencing the maintenance period and damaging the aspect and the potential benefit of medicinal plants (McKee, 1995; Buckenhüskes and Rendlen, 2004).

Also medicinal plants and spices are contaminated by different contaminations during harvesting, handling, transportation and storage (Grecz *et al.*, 1986). Many researchers such as Abba *et al.* (2009), Abou Donia (2008), Abou-Arab *et al.* (1999), Aguilera *et al.* (2005), Anotonia *et al.* (2010), Nagy *et al.* (1998), Banerjee and Sakar (2003), Chomnawang *et al.* (2003), Hashem and Alamri (2010), Kocic-Tanackov *et al.* (2007), Kosalec *et al.* (2009), Sospedra *et al.* (2010), Mandeel (2005), Martins *et al.* (2001), Tournas and Katoudas (2006), studied the microbial load of medicinal plants and spices and they reported the presence

of different contamination including pathogenic bacteria such as *Staphylococcus aureus*, *Shigella* spp., *Salmonella*, *Escherichia coli*, *Clostridium perfringens*, fungi, molds, mesophilic aerobic bacteria (total count) and Enterobacteriaceae.

The aim of this study was to investigate the microbial load of important medicinal plants in Iran including zataria, summer savory, mint, balm and Indian valerian. Also, the microbial load in aqueous extract, alcoholic extract and brewing of these plants were evaluated.

Materials and methods

Sample collection

A total of 44 dried samples including Indian valerian, zataria, summer savory, mint, balm were collected randomly from traditional herbal shops in Mashhad city (Khorasan Razavi province). The samples (10 g) were transferred to the laboratory of Microbiology in the Department of Biology, Faculty of Sciences of Ferdowsi University of Mashhad for microbial load evaluation and each sample of these plants species was evaluated.

The brewing and extraction preparation of the medicinal plants

The saline buffer (8.5 g NaCl, 1 g peptone) was added to 1 L hydro and heated until it reaches the boiling point. At a ratio of 1:10, the samples of mint, zataria and Indian valerian were mixed with the saline buffer and shaken (100 rpm) for 20 minutes. Hydro extracts were made by dried plants hydro-distillation (1:10, 90 ml hydro-distillation mix were added to 10 g dried sample). Hydro alcoholic extract was made by 10 ml of 96% alcohol ethanol with 90 ml hydro-distillation and it was blended (10:1) with 10 g of dried plants' samples and it was shaken (100 rpm) for 24 hours.

Microbiological study

The 25 g sample of medicinal plants were weighed and diluted with 225 ml buffered peptone hydro after which it was homogenate for 2 min; the homogenate was used for determination of the microbial load. Enterobacteriaceae and Coliform were determined using 0.1 ml of each dilution in VRBL agar and VRBG agar, over-layered with VRBL agar/VRBG agar, thus the plates were incubated at 37 °C for 24 h (according to the method of ISO 4832, ISO 21528-2). According to ISO 7954, YGC agar was used for determination of mold and yeast. The total count (plate count agar) was determined with the method of ISO 4833. Each sample was analyzed in 3 replicates (each replicate was tested with duplicate plates). To isolate the *Escherichia coli* used from chrome agar, the plates were incubated at 37 °C for 48 h (Soriano *et al.*, 2002). *Staphylococcus aureus* was evaluated using Baird-Parker agar (BP) containing egg-yolk tellurite emulsion (according to standard method ISO 6888-1). For isolation of *Salmonella* spp. (after standard method ISO 6579) were used Rappaport Vassiliadis broth (RV) and Tetrathionate Broth (TB) then XLD agar and BSA Agar.

Statistical analysis

All tests were performed in three replications. Analysis of variance and means comparison were calculated, by using SAS 9.1. Means square comparisons were different at the 5% significance level by the Least Significant Difference test.

Results

The results of microbial load analysis are summarized in Tabs. 1 and 2. The highest total count was found in Indian

valerian, with the mean of 6.36 log cfu/g and the lowest of total count was found in zataria samples, with the mean of 4.86 log cfu/g. All medicinal plant samples, except zataria and Indian valerian, were contaminated by total count. Except zataria, one hundred percent of Indian valerian, summer savory, mint, balm were contaminated by mold and yeast. The highest and the lowest of mold and yeast count were observed in Indian valerian and zataria, respectively. One hundred percent of the summer savory, mint, balm, 44% of the zataria, 88% of the Indian valerian, samples showed Enterobacteriaceae contamination. The highest and the lowest of Enterobacteriaceae contaminations were observed in Indian valerian (5.95 log cfu/g) and balm (4.36 log cfu/g) samples, respectively. All samples of balm and summer savory were contaminated by Coliform. The highest of Coliform (5.60 log cfu/g) was observed in mint and the lowest in zataria (3.25 log cfu/g) samples.

The results showed that the medicinal plant samples were contaminated by total count (100%), Enterobacteriaceae (85%), Coliform (83%), mold and yeast (98%) and *E. coli* (2.27). Indian valerian and zataria had the highest and the lowest of the microbial load. Among the studied medicinal plants, all samples of balm and summer savory were contaminated by total count, mold and yeast, Enterobacteriaceae and Coliform. All 44 tested medicinal plants were free from the *Salmonella* spp. and *Staphylococcus aureus* pathogenic bacteria.

Analysis of variance (Tab. 3) of the microbial load in the extract of summer savory, balm, mint, zataria, Indian valerian and mint showed that contamination rate had some significant differences. Data in Fig. 2 display the microbial load of summer savory affected by hydro and hydro alcoholic extract. Thus no mold and yeast, nor Coliform were detected in hydro and hydro alcoholic extract and the total count of this herb decreased in hydro and increased hydro alcoholic

Tab. 3. Variance analysis of the microbial load of the studied medicinal plants extract

Source of variation	Df	Mean Square		
		Total count	Mould and yeast	Coliform
Extract	2	8.2414**	34.6853**	42.2572**
Herb	4	6.9955**	12.5903**	8.8783**
Extract × herb	8	3.4723**	3.1720**	4.6912**
Error	30	0.0309	0.3853	0.6641

** Significant at 1% level

Tab. 1. Mean (M), range (R) and incidence (I) of total count and mould & yeast of the studied medicinal plants

Herbal name	Total count (log cfu/g)			Mould & yeast (log cfu/g)		
	M	R	I	M	R	I
Mint	6.06	5.30-6.64	100%	5.54	4.69-5.96	100%
Balm	5.45	4.47-5.69	100%	4.30	3.77-4.60	100%
Summer savory	5.09	4-5.35	100%	4.32	3.77-4.74	100%
Zataria	4.86	2.69-5.70	100%	3.73	2.50-4.30	89%
Indian valerian	6.36	5.90-6.47	100%	6.15	5.34-6.46	100%

Tab. 2. Mean (M), range (R) and incidence (I) of Enterobacteriaceae and Coliform of the studied medicinal plants

Herbal name	Enterobacteriaceae (log cfu/g)			Coliform (log cfu/g)		
	M	R	I	M	R	I
Mint	5.44	4.60-5.87	100%	5.60	5.54-5.80	80%
Balm	4.36	4-4.60	100%	4.25	3.87-4.39	100%
Summer savory	4.94	4.30-5.20	100%	5.19	4.30-5.47	100%
Zataria	5.35	4.69-5.47	44%	3.25	2.60-3.60	56%
Indian valerian	5.95	5.47-6.17	88%	5.30	4.56-5.55	88%

extract, respectively (Figs. 1, 2, 3). No mold and yeast, nor Coliform were detected in hydro and hydro alcoholic extract in zataria; also the total count was not observed in hydro alcoholic extract, while total count had no significant decrease in hydro extract with comparison to the control. No significant difference was observed between the hydro and hydro alcoholic extract of Indian valerian in the total count of microorganisms, but the values were significantly lower as compared to the control mold and yeast, that were not significantly differentiated among experimental treatments. No Coliform was showed in hydro extract of Indian valerian, and no significance was showed on Coliform of Indian valerian between alcoholic extract and the control. Results showed that Coliform and mold and yeast had low values in hydro and

hydro alcoholic extract of balm, while total count is slightly increasing in hydro extract of balm. The results indicated that no Coliform was found in hydro alcoholic extract of mint, also no mold and yeast was found in hydro and hydro alcoholic extract of this herb. Significant decrease was indicated on Coliform and total count in hydro extract and hydro alcoholic extract of mint, respectively. The main increase was detected on total count in hydro extract of mint.

Mean squares comparison of the microbial load of the studied medicinal plants extracts (Tab. 4) showed that the highest and the lowest of microbial load were observed in control and hydro extract of medicinal plants respectively. Results indicated that the highest and the lowest of contamination were observed in balm and zataria (Tab. 5).

Results obtained by the analysis of variance of the microbial load in the brewing of the studied medicinal plants samples are shown in Tab. 6 ($p > 0.01$).

According to Figs. 4 and 5, the total count of Coliform, mold and yeast in the brewing of zataria indicated significant difference in comparison to before brewing data, so that all 3 contaminants were not observed in the brewing of this

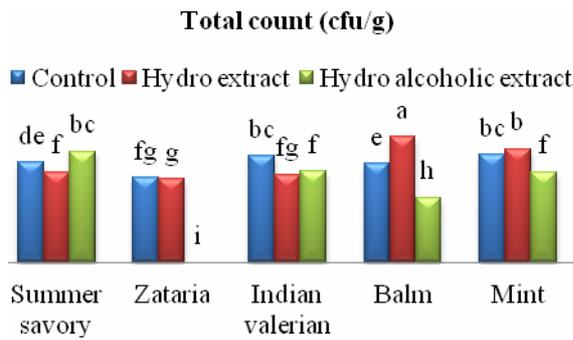


Fig. 1. Mean comparison of total count, in hydro and hydro alcoholic extract

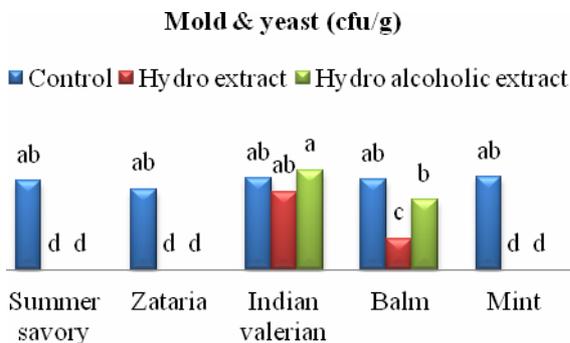


Fig. 2. Mean comparison of mould and yeast in hydro and hydro alcoholic extract

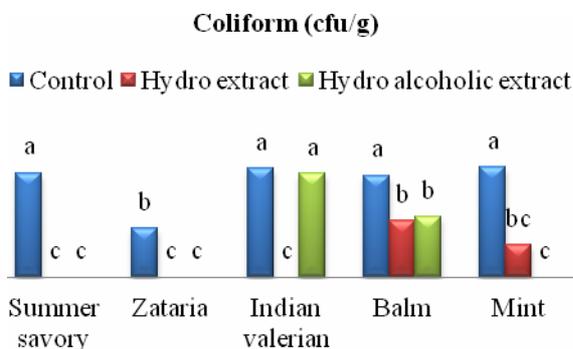


Fig. 3. Mean comparison of Coliform in hydro and hydro alcoholic extract

Tab. 4. Mean squares of microbial load of the studied medicinal plants extracts

Treatment	Total count	Mould and yeast	Coliform
Control	4.4947 ^a	3.7868 ^a	3.8994 ^a
Hydro extract	4.5200 ^a	0.9333 ^c	2.7333 ^b
Hydro alcoholic extract	3.2236 ^b	1.4488 ^b	1.3503 ^c

Means followed by the same letter in a column are not significantly different using LSD test at $p=0.05$

Tab. 5. Mean squares of microbial load of the studied medicinal plants

Herbal name	Total count	Mould and yeast	Coliform
Balm	4.3611 ^b	2.7234 ^b	3.0088 ^a
Indian valerian	4.2893 ^b	3.8309 ^a	2.9098 ^a
Mint	4.6855 ^a	1.3175 ^c	1.9530 ^b
Summer savory	4.5346 ^a	1.2596 ^c	1.4337 ^{bc}
Zataria	2.5266 ^c	1.5202 ^c	0.6667 ^c

Means followed by the same letter in a column are not significantly different using LSD test at $p=0.05$

Tab. 6. Variance analysis of microbial load in brewing medicinal plants samples

Source of variation	Df	Mean Square		
		Total count	Mould and yeast	Coliform
Brewing	1	4.0707 ^{**}	27.7760 ^{**}	51.8840 ^{**}
Herb	2	4.8163 [*]	3.6034 ^{**}	0.0048 ^{ns}
Brewing × herb	2	2.8631 ^{**}	3.4679 ^{**}	0.0048 ^{ns}
Error	12	0.4572	0.1279	0.0136

** , * significant; ns = non-significant, respectively at $p < 0.01$

herb. In the brewing of Indian valerian, a decrease of mold and yeast and a slight increase on the total count, as well as no Coliform, were noted. For mint, the brewing was effective on Coliform, mold and yeast, while no effect was noted on the total count.

According to the mean comparison (Tab. 7), the microbial load was lower in the brewing compared to before brewing.

Among all plants studied, zataria had the lowest microbial load, while mint and Indian valerian had the highest of microbial burden (Tab. 8).

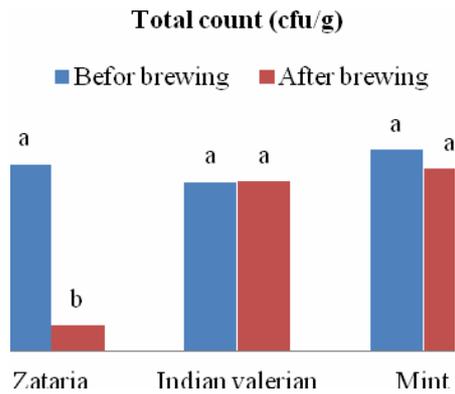


Fig. 4. Mean comparison of total count in brewing

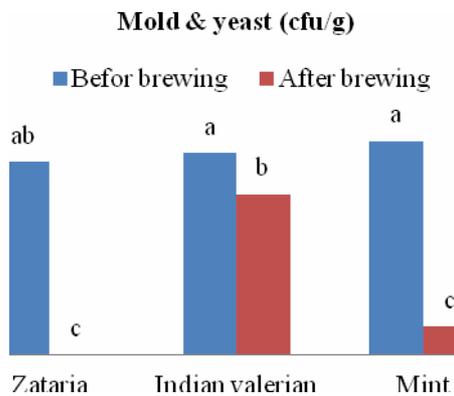


Fig. 5. Mean comparison of mould and yeast in brewing

In this study were observed various microbes in samples of mint, Indian valerian, balm and summer savory; samples of Indian valerian and mint had high levels of microbes; as this can be related to different culture conditions, production and packaging (Sospedra *et al.*, 2010). Results indicate that hygienic conditions must be improved in various stages of production, packaging and transport. *Escherichia coli* was found in one sample of mint. Banerjee and Sarkar (2003) found *E. coli* in garlic, while Friedman *et al.* (2002) had shown that *E. coli* and *Salmonella* were present in cinnamon. Schweiggert *et al.* (2007) found that technology differences from growth stages to final production of medicinal herbs are effective in

Tab. 7. Mean comparison of microbial load in before and after brewing

Treatment	Total count	Mould and yeast	Coliform
Before brewing	3.6978 ^a	3.5978 ^a	3.3955 ^a
After brewing	2.7467 ^b	1.1133 ^b	0.0000 ^b

Means followed by the same letter in a column are not significantly different using LSD test at p=0.05

Tab. 8. The average comparison of microbial load of the studied medicinal plants

Herbal name	Total count	Mould and yeast	Coliform
Zataria	2.2133 ^b	1.7100 ^b	1.5950 ^b
Indian valerian	3.5883 ^a	3.2150 ^a	1.7350 ^{ab}
Mint	3.9250 ^a	2.1417 ^a	1.7633 ^a

Means followed by the same letter in a column are not significantly different using LSD test at p=0.05

contamination level. In the current study, the highest level of contamination was observed for Indian valerian; it might be due to unsuitable growth medium, manipulation and packaging. Variant levels of high microbial burden were also found in the mint samples. Contamination of zataria was lower than others in the present study. Zataria had the lowest total count, mould and yeast in brewing compared to mint and Indian valerian.

Sospedra *et al.* (2010) reported that bacterial contamination was not observed in basil, ginger, thyme, dried parsley and cloves. Chen *et al.* (2008) also reported that ginger was free of bacteria, while Lachowicz *et al.* (1998), Montes *et al.* (1998) and Sağdıç (2003) demonstrated that basil, thyme and oregano had antimicrobial effect. Tourans and Katsoudas (2006) observed that ginseng extract was not contaminated with molds and yeasts, but 50% of the ginseng dry samples investigated were contaminated with bacteria.

The microbial contamination limit was determined for spices by the Institute of Standards and Industrial Research of Iran. According to this standard, total count in summer savory, balm and zataria was within the national standards, but microbial load of the other plants studied were not accordance to these limits.

After brewing the microbial load decreased in the studied medicinal plants. Brewing had high effect on mould and yeast in zataria, Indian valerian and mint. Roggentin *et al.* (2005) investigated the effect of brewing temperature and brewing period, on microbial activity of herbal infusions. The results showed that herbs need boiling water for brewing, because water with lower temperature cause an increase in the number of microorganisms of the brewing. Arthur *et al.* (2011) evaluated steam pasteurization and temperature treatments (99 °C for 5 min) on the microbial load of *Lippia multiflora* leaves. Results showed that pasteurization treatments reduced the microbial load and no Coliform was observed.

Conclusion

In this research, a variety of microbial load was observed in several medicinal plants. Therefore, hygienic conditions should be improved in different stages of cultivation, harvest, transfer, processing and packaging of medicinal plants. These results shows that, considering the importance of medicinal plants in the human health and the large usage of the medicinal herbs in various forms for disease prevention and treatment, culture, harvest, transfer and processing, of these crops should be done in sanitary conditions. Thus there is a need of environmental conditions control and improvements in hygiene procedures during production and processing of herbs. A suitable decontamination method for disinfection before and when packaging medicinal plants would be recommended.

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