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Identification and Quantification of Microbial Contaminations Present in Herbal Medicines Commonly Consumed by Women in Riyadh, Saudi Arabia

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Abstract

The present study aimed to investigate the microbial populations such a bacteria and fungi contamination present in the herbal medicinal preparations (*Lepidium sativum*, *Nigella sativa*, *Cuminum cyminum*, *Foeniculum vulgare*, *Pimpinella anisum*, *Trigoneela foenum-graecum*, *Cinnamomum verum*, peel coffee, *Alchemilla valgaris*, *Vitex agnus-castus*) commonly available in different parts of Riyadh, Saudi Arabia. To determine the contamination of the herbal products, experiments such as total aerobic bacterial plate counts were evaluated by various plating techniques. The isolation and identification of fungi were done using czapek dox agar and potato dextrose agar. The results showed that a total of herbal remedies were contaminated with bacteria and fungi (100%). Among the contaminated products, peel of coffee and *Vitex angnus-castus* were noted more contamination than the other herbal medicinal plants. The results confirmed that the traditionally prepared herbal medications in Riyadh city are likely to be contaminated with a wide variety of potentially pathogenic bacteria and fungi. Therefore, before consumption, the quality assurance of these products should be thoroughly enforced and monitored in the production and distribution of herbal preparations. In conclusion, the present study gives proper evidence that the consumed herbal products contain different levels of pathogenic microbes.

Keywords

Traditional Medicinal Plants, Bacterial Contamination, Fungal Contamination, Identification

1. Introduction

Postpartum period has been influenced by multiple cultural beliefs and practices transmitted from generation to generation. Some traditional practices are beneficial to the mother and baby, whereas others are not. Therefore it is essential for planning and implementing health education programs for these women to realize the beneficial and harmless practices. Postpartum period is the period beginning immediately after the birth of a baby and extending for about six weeks. It is the time during which the mother's body including hormonal levels and general and reproductive systems returns to a non-pregnant state [1]. Postpartum period is one of the most important stages for the mother-child dichotomy, and has been influenced by multiple cultural beliefs and practices transmitted from generation to generation. Internationally, many studies described the traditional beliefs and practices surrounding child bearing process. Some traditional practices are beneficial to the mother and baby, whereas other practices are not [2] [3] [4]. Traditional postpartum beliefs and practices are common in many countries. Among that, one common belief in many non-Western cultures is the necessity of maintaining a "hot-cold balance" within the body after the birth of a baby. Accordingly, in some cultures, traditional midwives emphasize the application of heat in the postpartum period. New mothers are instructed to use heated water to preserve their warmth by taking the herbal bath, according to the region. They believed that a hot bath increases the flow of milk, and prevents breast milk from becoming "cold". The history of using herbal medicines is inextricably intertwined with that of modern medicine [5]. Many synthetic drugs listed as conventional medication were originally derived from plants. Traditional herbal plants use various herbal preparations to treat various types of ailments, including diarrhea, urinary tract infections, typhoid fever and skin diseases [6] [7] [8]. Most of the herbal preparations are used in different forms and may carry a large number of various kinds of microbes originating from soil usually adhering to leaves, stems, flowers, seed and root of the herbs [9] [10] [11]. The World Health Organization (WHO 1998) survey indicated that about 70% - 80% of the world population particularly in developing countries relies on non-conventional medicines mainly of herbal origins for their primary health care [12]. This is because herbal medicines are accessible and cheap [13] [14]. Therefore, the quality and safety of herbal preparations are also of great concern. The WHO (1993) explained that quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of utmost importance [12]. It has been showed that the quality criteria for herbal drugs are based on a clear scientific definition of the raw materials. It is difficult to establish comprehensive quality criteria for herbal drugs due to "professional secrecy"' of herbalists, but in order to improve the purity and safety of the products, observation of basic hygiene during preparation, standardization of some physical characteristics such as moisture content, pH and microbiological contamination levels is desirable. Previous studies have confirmed the presence of potential contami-

nants in herbal preparations [15]. The contaminants that present serious health hazard are pathogenic bacteria such as *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp. and other Gram positive and Gram negative strains of bacteria [16] [17]. Unfortunately, no researches (to the best of our knowledge) have been carried out to determine the microbiological safety of these herbal products. In this paper, the level of contamination of powdered herbal products marketed in Riyadh city with selected pathogenic bacteria and fungi was determined and also the antimicrobial susceptibility pattern of these identified microbial pathogens were determined.

2. Materials and Methods

2.1. Study Area and Sampling

A total of 10 different herbal preparations were purchased randomly from herbal shops in Riyadh city. Packaged herbal samples were collected and taken to the laboratory, while those that were not packaged (such as herbal preparations sold by local herbalist) were collected in sterile cans glass [18]. All samples collected from the sites were analyzed in the laboratories of Department of Biology, Prinses Noura University, Saudi Arabia.

2.2. Preparation of Culturing Media

All dehydrated media were prepared according to manufacturer's instructions. They were mixed with distilled water and dissolved by gentle heat to boil. The media were sterilized in an autoclave (LTE J7090 model, LTE Scientific Ltd., England) at 121˚C for 15 min. The sterile media were dispensed or poured into sterilized petri dishes and allowed to cool. The sterility of the prepared media was checked by incubation of blindly selected plates at 37˚C for 24 h.

2.3. Isolation and Identification of Bacteria

A stock solution of the each sample was prepared by weighing one gram (1 g) of the sample into 9 mL of sterile water and shaken thoroughly. A ten-fold serial dilution of the bacterial suspension was made. This was done until 10^{-4} dilution was achieved. 1 mL was then pipetted from the 10^{-4} dilution onto the surface of each of two inoculated onto blood agar and nutrient agar media and incubated aerobically at 37˚C for maximum up to 48 h, and repetition three times to ensure the counting of the bacterial colonies.

2.4. Isolation and Identification of Fungi

For the isolation of fungal strains 1 gram from each sample and inoculated onto two media czapek dox agar and potato dextrose agar and incubated at 25˚C for 4 days.

2.5. Data Statistical Analysis

The obtained data were statistically analyzed using the Analysis of Variance

(ANOVA) with one way with the MSTAT-C statistical package. The least significant difference procedure (LSD) was used at 0.05 level of probability.

3. Results and Discussion

The results of the moisture content showed that there was remarkable variation among the different herbal preparations sampled. European Agency for the Evaluation of Medicinal products (1998) suggested that water content should be included in the list of comprehensive specifications for herbal medicinal products especially the powdered forms [19]. The maximum moisture content limit of 8%/g of herbal preparations are satisfactory according to National Agency for Food and Drug Administration and Control (NAFDAC SOP 2000) [20]. In this study, (100%) of the 10 herbal products were within, while 5 (50%) were outside the NAFDAC stated limit. Even at less than 58% moisture content limit various pathogenic bacteria were found. The fungi counts observed in the (100%) herbal preparations with high moisture contents were high, suggesting that high moisture contents favored the growth of pathogenic bacteria as well as non-pathogenic ones in herbal preparations. Similarly, the low bacterial counts in the other preparations could be attributed to very low moisture contents. In order to ascertain the correlation between the moisture content and bacterial load, Pearson chi-square correlation test was employed. At 0.01 level of confidence there was positive correlation between moisture content and bacterial counts (r = +0.109).

As shown in Table 1 and Table 2 and Figure 1, there were no statistically significant differences at the level of 0.05 or less for plant samples (*Lepidium sativum*, *Nigella sativa*, *Cuminum cyminum*, *Foeniculum vulgar*, *Pimpinella animus*, *Trigoneela foenum-graecum*, *Cinnamomum verum*, *Alchemilla valgaris*). As it is clear from the results shown in Table 1, there are no statistically significant differences at the 0.05 level or less about (peel coffee) variable depending on the study. It is clear from the results described above and no statistically significant differences at the level of about 0.01 or less (*Vitex agnus-castus*) variable depending on the study. In order to determine the direction of the differences between each of the two study groups towards the direction on these axes were used "LSD" test and the results were illustrated in Figure 1 and Figure 2.

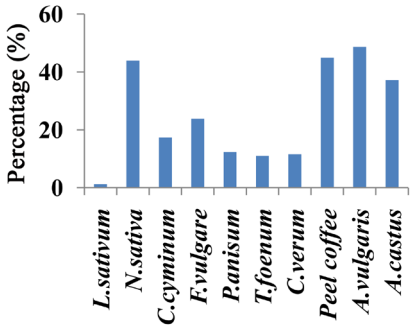

Figure 1. Percentage of the growth bacteria and fungi isolation from herbal medicine.

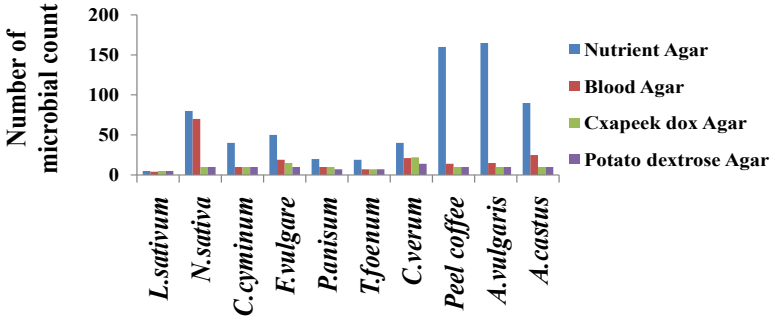

Figure 2. Range of the growth bacteria and fungi isolation from herbal medicine in different media.

Table 1. Average bacteria and fungi count of sample (×104 CFU/g).

		Sum of squares	df	Mean Square	F	Significance
A1	Between Groups	18.917	3	6.306	2.162	0.171
	With in Groups	23.333	8	2.917		
	Total	42.25	11			
A2	Between Groups	16,988.25	3	5662.750	2.725	0.114
	With in Groups	16,626.667	8	2078.333		
	Total	33,614.917	11			
A3	Between Groups	23.12.00	3	770.667	2.369	0.147
	With in Groups	2602.667	8	325.333		
	Total	4914.667	11			
A4	Between Groups	4387.000	3	1462.333	1.926	0.204
	With in Groups	6074.667	8	759.333		
	Total	10,461.667	11			
A5	Between Groups	196.00	3	65.333	0.547	0.664
	With in Groups	954.667	8	119.333		
	Total	1150.667	11			
A6	Between Groups	448.667	3	149.556	0.646	0.607
	With in Groups	1851.333	8	231.417		
	Total	2300.000	11			
A7	Between Groups	2593.667	3	864.556	2.673	0.118
	With in Groups	2587.333	8	323.417		
	Total	5181.000	11			
A8	Between Groups	53,020.250	3	17,673.417	4.524	0.039
	With in Groups	31,250.667	8	3906.333		
	Total	84,270.917	11			
A9	Between Groups	57,842.000	3	19,280.667	3.540	0.068
	With in Groups	43,572.667	8	5446.583		
	Total	101,414.7	11			
A10	Between Groups	22,177.000	3	7392.333	23.630	0.000
	With in Groups	2502.667	8	312.833		
	Total	24,679.667	11			

Table 2. Occurrence and frequency of fungi and bacteria with herbal medicine of four different media.

		LSD				95% confidence interval	
Dependant Variable	1 Q2	JQ2	Mean Difference (I-J)	Standard error	Significance	Lower Bound	Upper Bound
A8	Nutrient Agar	Blood Agar	150.33*	51.03	0.019	32.65	268.01
		Czapek dox Agar	155.00*	51.03	0.016	37.32	272.68
		Potato dextrose Agar	155.00*	51.03	0.016	37.32	272.68
	Blood Agar	Nutrient Agar	−150.33*	51.03	0.019	−268.01	−32.65
		Czapek dox Agar	4.67	51.03	0.929	−113.01	122.35
		Potato dextrose Agar	4.67	51.03	0.929	−113.01	122.35
	Czapek dox Agar	Nutrient Agar	−150.33*	51.03	0.016	−272.68	−37.32
		Blood Agar	−4.67	51.03	0.929	−122.35	113.01
		Potato dextrose Agar	0.00	51.03	1.000	−117.68	117.68
	Potato dextrose Agar	Nutrient Agar	−150.33*	51.03	0.016	−272.68	−37.32
		Blood Agar	−4.67	51.03	0.929	−122.35	113.01
		Czapek dox Agar	0.00	51.03	1.000	−117.68	117.68
A10	Nutrient Agar	Blood Agar	80.67*	14.44	0.001	47.36	113.97
		Czapek dox Agar	104.67*	14.44	0.000	71.36	137.97
		Potato dextrose Agar	104.67*	14.44	0.000	71.36	137.97
	Blood Agar	Nutrient Agar	−80.67*	14.44	0.001	−113.97	−47.36
		Czapek dox Agar	24.00	14.44	0.135	−9.30	57.30
		Potato dextrose Agar	24.00	14.44	0.135	−9.30	57.30
	Czapek dox Agar	Nutrient Agar	−104.67*	14.44	0.000	−137.97	−71.36
		Blood Agar	−24.00	14.44	0.135	−57.30	9.30
		Potato dextrose Agar	0.00	14.44	1.000	−33.30	33.30
	Potato dextrose Agar	Nutrient Agar	−104.67*	14.44	0.000	−137.97	−71.36
		Blood Agar	−24.00	14.44	0.135	−57.30	9.30
		Czapek dox Agar	0.00	14.44	1.000	−33.30	33.30

*The mean difference is significance at the 0.05 level.

The microbial populations were not significant with the medicinal plants such as (peel coffee) in (nutrient agar) and the medium in the (blood agar, czapek dox agar, potato dextrose agar) (Table 3). As it is clear from the results described

Table 3. Frequency of the fungi and bacteria isolated from peel coffee and *Vitex agnus-castus.*

Potato dextrose agar	Czapek dox agar	Blood agar	Nutrient agar	Total numbers	N	Different media	Samples
*	*	*	-	160 ± 14	3	Nutrient agar	peel coffee
		-		9.67 ± 1.3	3	Blood agar	
	-			5.00 ± 0.023	3	Czapek dox agar	
-				5.00 ± 0.15	3	Potato dextrose agar	
**	**	**	-	109.67 ± 7.5	3	Nutrient agar	*Vitex agnus-castus*
		-		29.00 ± 2.11	3	Blood agar	
	-			5.00 ± 0.11	3	Czapek dox agar	
-				5.00 ± 0.043	3	Potato dextrose agar	

LSD for fungus and bacteria; *interaction = 0.56 ($p \leq 0.05$); **interaction = 0.01 ($p \leq 0.01$).

above that there are statistically significant differences at the level of 0.01 between the average (*Vitex agnus-castus*) in (nutrient agar) and the average M in (blood agar, Czapek dox agar, (potato dextrose agar) to the average (peel coffee) in (nutrient agar), where he was the highest. The limits of bacterial contamination given in European pharmacopoeia as reported by [17] are: total aerobic bacteria (10^5 CFU/g), Enterobacteria and other Gram negative organisms (10^3 CFU/g). *Escherichia coli* and *Salmonella* should be absent in the consumed medicinal plants. However, the herbal products under study did not meet these specifications in most cases. The samples were contaminated to varying degrees with pathogenic bacteria. Herbal preparations were however free from bacterial contamination, All herbal medicine (100%) had bacterial counts in the range of 1.0×10^7 to 4.5×10^7 CFU/g; while six (70.07%) showed count between 5×10^7 and 8.5×10^7 CFU/g. The bacterial counts, in general, ranged between 1.0×10^7 and 1.8×10^8 CFU/g respectively. Of concern also is the level of contamination of herbal medicinal preparations by pathogenic Gram negative bacteria. Approximately 58.7% of the samples were contaminated by *E. coli*, which is an intestinal bacterium and is an indicator for faecal contamination, and 46.7% were contaminated by *Salmonella typhi*. Surprisingly, 65.7% of the samples were contaminated by *Staphylococcus aureus*, while (19.3%) were contaminated by *Shigella* spp. Modernization of health care could benefit from integrating aspects of traditional practices and plant use into healthcare modernization programmes through active involvement of local people. It would facilitate the implementation of culturally appropriate healthcare that respects traditional knowledge and contributes to bio-culturally sustainable development. In addition, there is a need for ethno-botanical research into the rich bio-cultural diversity of the ethnic groups in Asia as rapid assimilation with main stream culture increases. Research focusing on traditionally ignored subjects such as women's health care [21] [22]. In general, ethnobotanical studies often overlook the variety and rela-

tive importance of plants used in women's healthcare [23] [24], with a few notable exceptions [25] [26]. Research focusing on contamination of herbal medicinal when women use at the postpartum period and the efficacy of these treatments that are both ancient and wide spread, could provide insights that could help to augment and improve both local and Western postpartum care.

4. Conclusion

It is known that postpartum period is the most crucial period for the mother and the child. During this period, the immunity of the mother is enhanced and protected by the inhalation of various health benefits herbal medicines. In the present study, the microbial populations of various herbal medicines were determined. The study concluded that, the used herbal medicines contain different levels of microbial pathogens such as Gram Positive and Gram Negative bacteria together with filamentous fungi. However, different levels of public awareness should be made related to the importance of the plant species used such as different methods of plant collection, various preparation techniques, sterile processing techniques. Thereby the medicinal properties of the plants can be protected for a long time.

Conflict of Interest

We declare that we have no conflict of interest.

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