MICROBIAL CONTAMINATION IN HERBS

DARAKHSHAN ARA[1]*, HUMERA ANWER[2] AND RUBINA PARWEEN[2]

[1]Department of Basic Sciences, Mathematics and Humanities, Dawood University of Engineering and Technology Karachi, Pakistan.
[2]Department of Chemistry, Federal Urdu University of Arts, Science Technology, Gulshan-e-Iqbal Campus Karachi, Pakistan.
**, Corresponding author e-mail: ara_ahmed@hotmail.com*

Abstract

Pale catechu, Olibanum and Hyoscyamus niger are widely used as clinically as well as home remedy. This study was conducted to evaluate the microbial load in terms of colony forming units such as fungi and bacteria in the samples of selected herbs. In this regard, media culture Petri plate technique was employed and Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) were prepared for the fungal investigation while Nutrient Agar (NA) for the development of bacterial colonies. The total mycobiota comprised of five species namely *Alternaria solani, Aspergillus flavus, A. niger, Fussarium oxysporum* and *Neurospora cressa.* The samples of Bosvelia serrate exhibited the highest fungal diversity and lowest bacterial counts in contrast to other herbal samples. Among all isolated species, *A.niger* was recorded as least abundant whereas *Neurospora cressa* was found most abundant fungal specie.

Introduction

Herbs are widely taken in developing world culture as a home remedy for the treatment of many infections, injuries, wounds and for the various health care, usually taking directly or after applying semi protocols such as soaking, grinding etc. Unhygienic storage facilities, improper handling and sales infrastructure within the market create alarming situations particularly in the form of microbial contamination.

Styptics are medicinal herbs to prevent bleeding via contraction of blood vessels (Nergiz *et al*., 1992). In spite of the fact that styptics can transmit several diseases. The atmosphere of Karachi (back bone of Pakistan) is rich in microbes (fungi and bacteria), since their growth facilitated by open main holes, domestic and industrial sewage, dust and smoke and unhygienic environment (Rao *et al*., 2009).

Pale catechu is one of the variety of Acacia catechu, use for medicinal purposes (pale catechu get from medicinal part of gambier) and it is a dried aqueous extracts of young shoots and leaves (Vinod, 2007). Generally people apply it for serious injuries, dermatitis, as blood astringent and it also a remedy for throat, oral and gum disease (Alam *et al*., 2011). Olibanum and *Hyoscyamus* niger (Khurasani Ajwain) are also medicinal herbs and widely used as an astringent medicines and for many other purposes. On the exposure in open air, these styptics contaminated by existed environmental microbes and therapeutic belongings of these styptics influenced by microbes and pose worst effects on patients. It is fact that the airborne mycobiota consisted *Alternaria solani, Aspergillus niger, A. flavus and Fussarium oxysporum* fungi may transmit mycotoxins (Miller *et al*., 1998) and inhalation of mycotoxins such as, aflatoxins, zearalenone, tricothecenes and secalonic acid may change the immunological reaction of the lung tissues or source of other risks to human health (Gerberick *et al*., 1984). Bacteria as compare to other microbes successively colonize plants while they mature and it is well recognized that plant pathogenic bacteria cause many serious diseases of plants throughout the world (Vidhyasekaran *et al*., 2002) but smaller quantity than viruses or fungi and cause somewhat less damage and economic cost (Kennedy *et al*,.1980). In this scenario health threats along with these herbs cannot be ignorable. Therefore, the current study was conducted to investigate mycobiota and bacterial counts in medicinal herbs including Pale catechu, Olibanum and Hyoscyamus niger .

Materials and Methods

Sampling: Approximately 100g of three herb samples Pale catechu, Olibanum and Hyoscyamus niger were purchased and separately packed in polythene bags from shop of herbal physician of local market in summer season. Samples were brought to the Laboratory, manually cleaned, labelled and then preserved in air tight glass jar at room temperature. While, Pale catechu and Olibanum were crushed before preservation, sieved to desired size of particles about 50µm and 150 µm respectively.

Isolation of fungal colonies: The mycobiota of herbs was assessed using different media. Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) were prepared for the recovery of fungi from the samples. A little quantity about 0.1g sample was sprinkled on media in plates and incubated at room temperature (28-30^{o} C) for 8

to 12 days. The identification of growing colonies were done on the basis of micro and macro morphological features following the standard manuals (Domsch *et al*., 1980; Ellis, 1976).

For the recovery of bacterial colonies from the samples, Nutrient Agar (NA) was prepared. In this perspective, serial dilutions were prepared by dissolving 1g of each sample in 9 ml of sterile distilled water separately. An aliquots (1ml) of diluted samples inoculated on triplicates of media containing plates and incubated at 35º C for 24 h. The developed bacterial colonies were counted through colony counter.

Table 1. Isolation of fungal species and microbial counts (cfu g^{-1}) from herbs samples.

Herb	**Fungal species**	**Total fungal counts cfu g^{-1}**	**Range of bacterial counts cfu g^{-1}**
Olibanum	*Alterneria solani*	3.30×10^2	$1.0 \times 10^1 - 1.8 \times 10^1$
	Aspergillus niger	6.60×10^2	
	Fussarium oxysporum	6.60×10^2	
	Neurospora cressa	3.30×10^2	
Hyoscyamus niger	*A. flavus*	6.6×10^1	$3.2 \times 10^2 - 4.0 \times 10^2$
	A. niger	1.2×10^1	
	Neurospora cressa	6.6×10^1	
Pale catechu	*Alterneria solani*	3.30×10^2	$2.0 \times 10^2 - 2.8 \times 10^2$
	A. flavus	6.60×10^2	
	Neurospora cressa	Ucc*	

*: Uncountable colonies

Results and Discussion

The averages of colony forming units (cfu g^{-1}) of fungal spp. in samples of three different herbs Pale catechu, Olibanum and Hyoscyamus niger collected from Karachi were shown in Table 1. Total five fungal species were recorded in these herbs samples. The highest number of fungal species namely *Alternaria solani, Aspergillus niger*, *Fussarium oxysporum* and *Neurospora cressa* were isolated from the samples of Olibanum. However, *Aspergillus niger* and *Fussarium oxysporum* were shown same and higher number of counts in contrast to other species. Although, least bacterial contamination was noted in the samples of this herb. Omogbai and Ikenebomeh, (2013) reported the occurrence of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Rhizopus stolonifer* and *Fusarium solanii* and bacterial counts ranged from 1.1×10^1 to 4.8×10^2 cfu/g in the samples of herbal teas , sold in Benin City, Nigeria.

Three fungal species were determined in the samples of two herbs (Hyoscyamus niger and Pale catechu). The colonies of *A. flavus*, *A. niger* and *Neurospora cressa* were sighted in Hyoscyamus niger from which *A. flavus* and *Neurospora cressa* showed higher counts in contrast to *A. niger*. The prevailing of *A. flavus* and *A. niger* in atmosphere of the Karachi was also reported by Rao *et al*. (2011). Even the presence of *A. niger* in edibles like tea whitener in powder form in sachet packaging was also recorded by Perween *et al*. (2013). In comparison to the other herbal sample, Hyosyamusw niger showed lowest fungal but highest bacterial counts.

In Pale catechu, *A. solani, A. flavus* and *Neurospora cressa* were identified and was found highly contaminated by *Neurospora cressa* which was revealed by uncountable colonies (Table1). Further, second highest counts was exhibited by *A. flavus* and comparatively moderate bacterial contaminated. Although *A. flavus* recognized as major toxigenic or cancer causing fungi as it produces aflatoxins (D'Mello and Macdonald, 1997; Frisvad *et al*., 2005).

Among all isolated species, *A. niger* was recorded as least abundant whereas *Neurospora cressa* was found most abundant fungal specie. Another investigation reported that *Aspergillus* spp. was recorded as one of most abundant fungi in the samples of prepared powdered herbal medicine while, the herbs were collected from certain areas of Nigeria (Anyanwu, 2010).

The results of the study revealed that the use of these herbs as home remedy could be prolong the infections and ultimately cause other disease since it was observed that herbal remedies were not sterile, particularly aerial contamination risk is very high since herbs or medicinal plants are available in market in open air. To prevent from these contaminants special hygienic care must be taken like soak during boiling of water and kept in air tight tumbler.

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