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Original Research Article

MICROBIAL ASSESSMENT OF HERBAL DRUGS AVAILABLE IN LOCAL MARKET OF LAHORE

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ABSTRACT

The present work was carried out considering the increased use of herbal products as alternative medicines in Pakistan. It is necessary to set appropriate standards for microorganisms in herbal drugs in order to reduce the risks for consumers' health. The presence of fecal coliform and moulds represents a potential risk of contamination. The microbial contamination of herbal drugs both branded (Qarshi ghutti, Khamira gaozban, Surficol, Sadoori, Carmina, Sauline and Toot Siah syrup etc.) and unbranded was determined. Forty eight samples of herbal drugs were purchased from local market and were analyzed for total viable count, spore former (aerobic and anaerobic), mold, yeast and coliforms. Total viable count ranged from 1.1×10^5 - 9.3×10^5 /g. The aerobic spore former varied from 1-59/g. Moisture content varied from 4-20%. Non fecal coliform were present in most of the samples but fecal coliforms were also present in few samples. *Aspergillus*, *Mucor* and *Penicillium* species were predominant in most herbal drugs.

Key words: Herbal drugs, microbial assessment, moisture content

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INTRODUCTION

About 30% of the pharmaceutical drugs are still extracted from different plant materials (Gloria and Asuncion, 1994). These are source of very affordable and effective drugs. At present, most of the medicinal plants have been processed into dosage forms that are more convenient to use and more readily available than raw materials. W.H.O estimates that 80% of the world population use herbal medicines for some aspects of primary healthcare (Chan, 2003) . The increasing popularity of natural drugs made their use a Public Health problem due to the lack of

effective surveillance of the use, efficacy, toxicity and quality of these natural products (Bugno *et al.*, 2006). The adverse effects of long-term herbal use, adulteration with toxic compounds and contamination by pathogenic microbials or natural toxins like mycotoxins have been reported for herbal products and medicinal plants (Mandeel *et al.*, 2005, Rizzo *et al.*, 2004, Tassaneeyakul *et al.*, 2004). The concern over quality of these products is mainly due to their potential contamination, considering their natural origin. Natural drugs should meet the requirements of quality, safety and efficacy (Calixto, 2000). Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins (Mandeel *et al.*, 2005, Tassaneeyakul *et al.*, 2004).

The major sources of microbial contamination is from raw materials, handling during processing, storage and transportation. This may pose a serious problem to the consumers health as microbial contaminants may cause intoxication or other forms of illness (Nahrstedt *et al.*, 1996). The object of microbial examination of any food or food product is to look their freedom from fecal pathogens, toxic bacteria and high viable count and spore formers. These types of studies have been reported by workers (Ramaday and Elnaby 1962). *Coliform* organisms are members of *Enterobacteriaceae* which ferment lactose with production of gas within 48h at 35°C. Coliform were instituted as indicators of fecal pollution of water in the United States by the Public Health Service (Sneath *et al.*, 1986). The *coliform* group of bacteria includes all the aerobic and facultatively anaerobic, gram negative, non sporulating bacilli. The classical species of this group are *Escherichia coli* and *Aerobacterearogens*.

MATERIALS AND METHODS

Collection of samples

20 herbal drugs for oral use in human were selected based on microbial caused diseases. These drugs were obtained from local market. A total of approximately 100g of the sealed samples were placed in the sterilized plastic bags and stored at 4°C until needed. The samples and their prescribed uses along with their manufacturers are given in table 1.

Microbial Assesment

25g of herbal drug was shaken in 250ml distilled water. 1ml of sample was added to maconkey bottles containing 9ml of sterilized saline water. Ten fold serial dilutions were performed up to 10⁻⁵, in buffered peptone water (MERCK).

Enumeration of microorganisms was performed by pour plating method using Nutrient agar medium for bacteria and potato dextrose agar medium for moulds. Plates were incubated upside down at 37°C and 26 ± 1°C for bacteria and fungi respectively. After incubation, the microbial colonies were counted, recorded and the number of colony-forming units (c.f.u) per gram were calculated. Results were compared with US Pharmacopoeia, 2005.

Thermophilic spore formers

The nutrient agar medium was used for aerobic spore former and Brain Heart Infusion Agar for

anaerobic spore former. Spore formers were isolated by heat shock method. The plates were incubated at 37°C for 24-48h. The numbers of bacterial colonies were counted after 24-48h.

Detection of *Coliform*

The coliform organisms were determined by standard multiple tube fermentation technique (Alcala, 1990).

Table 1: Herbal drugs and their prescribed uses

Samples	Prescribed uses
Qarshi Ghutti	Medicines to evacuate the bowels of newborn babies and infants, constipation in children.
Surficol	Effective in cough, cold, bronchitis and catarrh.
Khamira Gaoziban	As a remedy for perplexity and restlessness, sore throat, cold and flu.
Johar joshanda	As a remedy for flu, cough, cold, catarrh, irritation of throat and fever.
Sharbat Toot Siah	Effective in swelling and pain of throat, hoarseness of voice and helps relieve swelling of throat in diphtheria.
Zanjabeen	Effective in hyperacidity, nausea and vomiting, digestive system and migraine.
Musaffine	Medicine used in blood purification, natural glow to skin and face, pimples, prickles and boils associated with blood disorders.
Sauline	As a remedy for irritation of throat.
Carmina	As a remedy to correct indigestion, liver disorders, dyspepsia and constipation.
Sadoori	Effective in cough and congestion of lungs, chronic and acute bronchitis, cold, catarrh and esthma.
Dawa Al-shifa	Medicine used in hysteria, restlessness and tension.
Safi	Medicine used to purify blood, spurs the system on to start elimination of the accumulated morbid matter, the root cause of all ailments, through the intestines, kidneys, and skin. Corrects digestive system, constipation and prevents pimples.
Maseehi (Ajmal)	Effective in tuberculosis.
Hab-e-Banafsha	Effective in pain of throat, flu and influenza.
Unbranded	As remedy for common cold and flu.
Unbranded	Medicine used in fever, sore throat and irritation of throat.

RESULTS AND DISCUSSION

The data of (table 2) shows moisture content of different herbal drugs. Moisture content varied from 6% (Qarshi ghutti) to 20% (unbranded samples). It was found that branded samples had low moisture content as compared to unbranded sample. High amount of moisture allows the growth of microorganisms at a higher rate. As the water activity (a_w) decreases, the growth of

microbes is slowed or prevented (Scott, 1957 and Troller, 1973). The growth of mold is dependent on temperature, water activity, atmosphere and substrate i.e., pH, nutrients and inhibitors. At a given water activity, the growth response will change if any of the factors is optimal (Christian,1963).The herbal drugs were found contaminated with both pathogenic and non-pathogenic microorganisms.

Table 2: Microbial examination of herbal drugs

No	Product	Manufacturer	Sporeformer (c.f.u /gm)		coliform		Fungi		Moistur content	Total viable count
			Aerobic	Anaerobic	Fecal	Non- fecal	Mold	Yeast		
1	Qarshi ghutti	Qarshi industries (Pvt.)Ltd. Pakistan	19	7	-	+	4	-	6	5.2x10 ⁵
2.	Surficol	Qarshi industries (Pvt.)Ltd. Pakistan	13	7	-	-	9	-	7	5.4x10 ⁵
3.	Khamira Gaozban	Qarshi industries (Pvt.)Ltd. Pakistan	58	32	-	+	-	-	9	6.2x10 ⁵
4.	Johar joshanda	Qarshi industries (Pvt.)Ltd. Pakistan	17	3	-	+	9	-	6	4.2x10 ⁵
5.	Sharbat Toot Siah	Qarshi industries (Pvt.)Ltd. Pakistan	9	6	-	-	-	-	6	2.1x10 ⁵
6.	Zanjabeen	Qarshi industries (Pvt.)Ltd. Pakistan	22	15	-	-	6	-	6	6.2x10 ⁵
7.	Musaffine	Qarshi industries (Pvt.)Ltd. Pakistan	40	28	-	-	6	-	9	3.5x10 ⁵
8.	Sualine	Hamdard Laboratries (waqf) Pakistan.	30	22	-	-	12	-	6	6.6x10 ⁵
9.	Carmina	Hamdard Laboratries (waqf) Pakistan.	45	32	-	+	-	-	5	2.0x10 ⁵
10.	Sadoori	Hamdard Laboratries (waqf) Pakistan	6	3	-	+	3	-	5	2.9x10 ⁵
11.	Dawa Al-Shifa	Dawa khana Hakeem ajmal khan (Pvt) Ltd. Pak.	9	5	-	-	12	-	4	1.5x10 ⁵
12.	Safi	Hamdard Laboratries (waqf) Pakistan	15	8	-	-	19	-	5	3.2x10 ⁵
13.	Maseehi Ajmali	Dawa khana Hakeem ajmal khan (Pvt) Ltd. Pak.	40	32	-	+	-	-	6	4.3x10 ⁵
14.	Hab-e-Banafsha	Dawa khana Hakeem ajmal khan (Pvt) Ltd. Pak	59	42	+	+	9	2	6	5.4x10 ⁵
15.	Khamira	Unbranded	42	32	+	+	9	-	20	9.3x10 ⁵
16.	Cough syrup	Unbranded	70	52	+	+	30	9	20	9.2x10 ⁵

The number of microorganisms were maximum (9.3 x 10⁵ c.f.u/g) in unbranded sample. This contamination may be due to unhygienic conditions during processing. The presence of contaminants and the lack of proper labelling and appropriate packaging in samples were clear

indications that the manufacturers did not comply with existing regulations on herbal drug production. The presence of mold and yeast indicate the potential for spoilage and mycotoxigenesis and causes the spoilage of herbal drugs (Mandeeel, 2005). They secrete enzymes such as lipases, amylases and proteases, which can degrade lipids, starch and proteins. The maximum fungal load (30 c.f.u/g) was observed in unbranded cough syrup. The genus *Aspergillus* was the most dominant genus followed by *Penicillium* (Figure 1). Our results are in agreement with other workers (Bugno *et al.*, 2006). These moulds have been reported to have ability to produce mycotoxins.

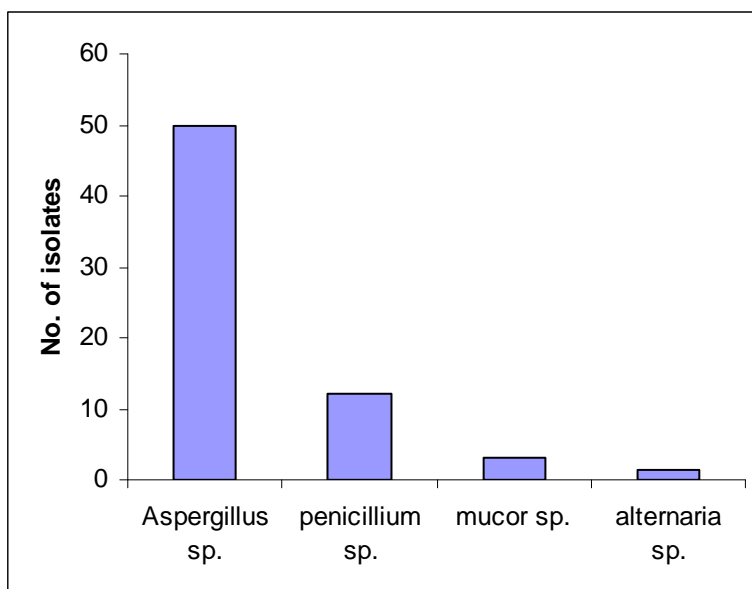


Fig 1: Mycoflora detected in herbal drugs

The non fecal coliform bacteria were present in most of the samples under study but the fecal coliform bacteria were present in all unbranded samples and one branded sample (Hab-e-banafsha). The presence of *Enterobacter* and *Escherichia coli* in drug samples not only indicated improper hygienic conditions in processing but it was also definitely contradictory to the claims of the manufacturer. The sample which is supposed to remedy and cure many health problems might be dangerous to the consumer's health. In most of the studies reported on the microbial contamination of food items, *coliform* bacteria have been common isolates (Robinson). Enteric disease caused by *coliform* bacteria result most likely from ingestion of material contaminated with these organisms (Stainer *et al.*, 1986). The presence of these potentially hazardous contaminant microorganism in seven of the sixteen drug samples was clearly hazardous to health since contaminant microorganisms alter the physical and chemical properties of materials they attack and may produce secondary metabolites like toxin. Moreover, these microorganisms are potential hazards themselves when ingested. There is therefore a high health risk in consuming herbal drugs unless regulatory agencies strictly require and monitor Good manufacturing Practices (GMP) among herbal drug manufacturers.

The data of (Table 2) shows aerobic spore former (in branded and unbranded samples).

Maximum aerobic (70/g) and anaerobic sporeformer (52/g) load was present in unbranded cough syrup (sample 16). This might be due to unhygienic conditions during processing. The presence of sporeformer might be a result of contamination of raw materials due to highly resistant spores, the bacteria are able to withstand adverse environmental conditions. It was found that population varied quite markedly in each of the samples.

CONCLUSION

Our results demonstrated that all of the samples exceeded the limit determined by the US Pharmacopoeia. This will open the way to investigate the raw materials, the processing procedure and general hygienic conditions under which drugs packed. Broadly speaking such marked change in bacterial counts among different samples may be due to unclean utensils and containers, particularly place and atmosphere where herbal drugs were packed.

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