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ESTIMATION OF MICROBIAL CONTENT OF HERBAL DRUGS AVAILABLE IN GWALIOR-CHAMBAL REGION

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ABSTRACT

Herbal remedies are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds. These products have the potential of contamination with different microorganisms. This is due to raw materials contamination and unhygienic production conditions. The present work was carried out considering the increased use of herbal products as alternative medicines in Madhya Pradesh. 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 molds represents a potential risk of contamination. The microbial contamination of herbal drugs both branded and non-branded was determined. Fifteen different herbal solid dosage forms were purchased randomly from identified herbal shops and retail outlets indifferent parts of Gwalior-Chambal Division and were analyzed for percent moisture content, total viable count, spore former (aerobic and anaerobic), mold, yeast and coliforms. Total viable count was found in the samples that may be hazardous if used. The aerobic and anaerobic spore former varied from 1-47/g. Moisture content varied from 6-22%. Non fecal coliform were present in most of the samples but fecal coliforms were also present in few samples. Many aerobic and anaerobic species were predominant in most herbal drugs samples.

Keywords: Herbal drugs, microbial assessment, moisture content.

INTRODUCTION

Herbal drugs are crude preparations of various kinds of medicinal plants. In other words, herbal drug is a dried medicinal plant, or any part thereof, such as leaf, stem, root, flower or seed. Herbal medicine has a long history, probably extending over 2000 years and is quite popular with many people [1]. Crude drugs and herbal medicines play an important role in home health care, health improvement, as alternative medicine and materials for medical products in many countries [2]. The use of medicinal plants is continually expanding worldwide. The increasing search for therapeutic agents derived from plant species is justified by the emergence of diseases, yet without proper treatment, and the growth of scientific knowledge about the herbal medicines as important treatment alternatives. The popularity of natural products is increasing day by day. Natural products do not have any harmful side effects.

Natural health products do not give immediate relief but they treat the cause of disease. Therefore, the quality and safety of herbal preparations are also of great concern [3]. 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. WHO estimates that 80% of the world population use herbal medicines for some aspects of primary healthcare [4]. 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. Most raw materials for pharmaceutical products support some form of microbial growth, depending on the nutritive properties and moisture contents. Hence, dry powder or tablets are capable of undergoing some form of microbial spoilage or degradation. The more serious problem of microbial contamination of herb is where there are no obvious signs of spoilage. Hence, it is usually advisable to have knowledge of the microbial contents of all drugs and medicines, whether they are required to be sterile or non-sterile [5]. The quality control of crude drugs has been at the discretion of each pharmaceutical company; therefore, microbial contamination level varies drastically from company to company. Currently, microbial contamination on crude drugs has become an issue and certain quality assurances have been sought from the good manufacturing practices stand-point. The major sources of microbial contamination are from raw materials, handling during processing, storage and transportation. Therefore it is necessary to estimate the microbial contamination level on crude drugs at each manufacturing stage. The concern over quality of these products is mainly due to their potential contamination, considering their natural origin. The microbial quality of pharmaceuticals is influenced by the environment and quality of the raw materials used during formulation. Natural drugs should meet the requirements of quality, safety and efficacy. 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 [6,7]. The contaminants that present serious health hazard are molds, yeast, pathogenic bacteria such as *Salmonella, Escherichia coli, Staphylococcus aureus*, *Shigella* species and other Gram positive and Gram negative strains of bacteria [8-11]. This may pose a serious problem to the consumers health as microbial contaminants may cause intoxication or other forms of illness. Fungal contamination has been reported to affect the chemical composition of the raw materials and thereby decrease the medicinal potency of herbal drugs. The most prominent fungal toxins reported are aflatoxins, zearalenone, ochratoxin and patulin, which are collectively known to cause hazards to the liver, nervous system, muscular system, respiratory organs as well as digestive and genital systems [12]. Based on this fact, we demonstrated the bacterial and fungal population.

MATERIALS AND METHODS

Materials

All cultures media (fluid soybean-casein digest medium, soybean casein digest agar medium, sabouraud dextrose broth, Vogel- Johnson agar medium, manitol-salt agar medium, cetrimide agar medium, fluid lactose medium, mac-conkey agar medium, selenitecystine medium, bluidtetrationate medium, brilliant green agar medium, bismuth sulfite agar medium, triple sugar-iron agar medium, sabouraud dextrose agar) and chemicals (potassium tellurite, glycerin, potassium iodide, iodine, brilliant

green) were purchased from Nesco chemical company, Gwalior. All materials and chemical reagents were of analytical grade. Experiments were done carefully with appropriate controlled room temperatures, humidity and other environmental conditions.

Collection of samples

Fifteen different herbal solid dosage forms (herbal tablets or herbal powders) of different brands were purchased randomly from identified herbal shops and retail outlets indifferent parts of Gwalior-Chambal Division. 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 polythene bags [13]. Approximately 100g of the sealed samples of each herbal drug were placed in the sterilized plastic bags and stored at 4°C for further study or requirement. All samples collected from the sites were analyzed in the laboratories of Department of Microbiology, Department of Pharmacognosy and Drug Development of Gurukul Institute of Pharmaceutical Science and Research, Gwalior and School of Studies in Pharmaceutical Sciences, Jiwaji University, Gwalior, Madhya Pradesh, India.

Preparation of samples

Handling of solid dosage forms for microbiological analysis was carried out according to standard procedures. All solid dosage form samples were powdered. A portion of each sample (10 g) was dispersed in fluid soybean-casein digest medium to make 100 ml in the aseptic conditions, clean rooms, areas and equipment [14].

Physical analyses

Determination of pH

The pH of the products was determined by using Digital pH meter. Sample solution (10%) was prepared by weighing 10g of the sample into 200ml beaker and adding sterile distilled water (100ml) with shaking to obtain a homogenous solution. There after the pH of the preparation was taken with Digital pH meter [15].

Determination of moisture content

A Mettler Toledo moisture analyzer was used to determine the moisture content of the powdered herbal preparations. One gram (1g) was weighed into a clean pan (which was a part of the machine accessories). The analysis was done automatically and the reading was shown in percentage on the screen [16].

Bioburden determination

The collected samples of herbal products were subjected to the following examinations: total viable count (TVC) by plate and multiple tube methods and presence or absence of *S. aureus, P. aeruginosa, E. coli, Salmonella sp. and C. albicans*. 10 g of each sample was suspended in appropriate medium like fluid lactose medium, mac-conkey agar medium. The total volume was adjusted to 100 ml by adding soybean-casein digest medium for detection of bacteria and sabouraud dextrose broth for detection of molds and yeasts. Aerobic bacterial colony counts were made by the pour plate technique on soybean casein digest agar. Plates were incubated in duplicate at 37°C for 48 – 72 h. After incubation, the number of colonies was recorded for each plate. Arithmetic mean counts were derived from each item having from 30 to 300 colonies per plate. On the other hand multiple-tube method based on United State Pharmacopeia (USP30) for detection of total aerobic count was carried out. Following the incubation period, by examining the tubes for growth, the most probable

number of microorganisms per gram of solid dosage forms specimens was expressed by reference to related table in USP30.

RESULTS AND DISCUSSION

Table 01 shows the percent moisture content and pH of all herbal drug samples. Moisture content varied from 6% to 22% (unbranded samples). It was found that branded samples had low moisture content as compared to unbranded or mixed herbal sample. High amount of moisture allows the growth of microorganisms at a higher rate. As the water activity decreases, the growth of microbes is slowed or prevented. The pH analyses in table 01 indicated that the herbal drug samples were within the pH ranges of 3 to 8. The growth of mold is dependent on temperature, water activity, atmosphere and substrate i.e., type of nutrients and inhibitors.

The herbal drugs were found contaminated with both pathogenic and non-pathogenic microorganisms. On the basis of colony appearance, Salmonella was found to be commonly present in all samples examined. The suspected colonies were transferred to the specific culture media as described in USP 30 and the plates examined and compared with the colony characteristics listed in USP 30. Arithmetic mean counts were derived from each item having from 30 to 300 colonies per plate. The presence of *S. aureus, P. aeruginosa, E. coli and C. albicans* were not observed in some samples. The morphologic characteristics of colonies on the surface of brilliant green agar medium and bismuth sulfite agar medium confirmed the presence of salmonella species in all herbal solid dosage forms samples. Also, the inoculated butt-slant tube of triple sugar-iron agar medium confirmed the presence of Salmonella species in all samples.

The number of microorganisms was maximum in unbranded sample. This contamination may be due to unhygienic conditions during processing. They secrete enzymes such as lipases, amylases and proteases, which can degrade lipids, starch and proteins.

Table 1. Percent Moisture content and pH of herbal drug samples

Sr. No.	Samples	Moisture content	pH
1	Sample 1 (*Phyllanthus emblica*)	7%	6
2	Sample 2 (*Matricaria chamomilia*)	9%	5
3	Sample 3 (*Anethum graveolens*)	8%	6
4	Sample 4 (*Ammi visnaga*)	9%	5
5	Sample 5 (*Justicia adhotoda*)	7%	7
6	Sample 6 (*Boswelia carterii*)	6%	8
7	Sample 7 (*Trigonella foenum*)	8%	6
8	Sample 8 (*Thymus serphyllum*)	6%	7
9	Sample 9 (*Panax ginseng*)	7%	6
10	Sample 10 (*Ginkgo Biloba*)	9%	6
11	Sample 11 (*Terminalia chebula*)	7%	6
12	Sample 12 (*Sphaeranthus indicus*)	9%	5
13	Sample 13 (*Achyranthes aspera*)	6%	7
14	Sample 14 (Unbranded; Mixed herbs)	19%	3
15	Sample 15 (Unbranded; Mixed herbs)	22%	4

Table 2. Microbial examination of herbal drug samples

Sr. No.	Samples	Microbial contamination		Total Viable Count
		Aerobic	Anaerobic	
1	Sample 1 (*Phyllanthus emblica*)	12	6	$2.1x10^5$
2	Sample 2 (*Matricaria chamomilia*)	11	5	$3.4x10^5$
3	Sample 3 (*Anethum graveolens*)	9	3	$2.3x10^5$
4	Sample 4 (*Ammi visnaga*)	21	8	$4.1x10^5$
5	Sample 5 (*Justicia adhotoda*)	5	2	$1.8x10^5$
6	Sample 6 (*Boswelia carterii*)	21	1	$3.9x10^5$
7	Sample 7 (*Trigonella foenum*)	12	10	$2.0x10^5$
8	Sample 8 (*Thymus serphyllum*)	15	9	$3.6x10^5$
9	Sample 9 (*Panax ginseng*)	19	10	$4.5x10^5$
10	Sample 10 (*Ginkgo Biloba*)	11	6	$2.0x10^5$
11	Sample 11 (*Terminalia chebula*)	19	9	$4.3x10^5$
12	Sample 12 (*Sphaeranthus indicus*)	18	7	$2.6x10^5$
13	Sample 13 (*Achyranthes aspera*)	21	11	$3.1x10^5$
14	Sample 14 (Unbranded; Mixed herbs)	39	19	$6.3x10^5$
15	Sample 15 (Unbranded; Mixed herbs)	47	33	$9.9x10^5$

CONCLUSION

The results in this study showed that the microbial load of the herbal products varied considerably. The samples were contaminated to varying degrees with bacteria and fungi. In case of individual product, most of them met with the given microbiological standard but few of them could not pass the entire test. However, this study gave emphasis on the fact that manufacturers should ensure the lowest possible level of microorganisms in the raw materials, finished dosage forms and the packaging components to maintain appropriate quality, safety and potency of the medicines. Quality has to be built throughout the process beginning from the selection of propagating materials to the final products reaching to the consumers. 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. All drugs are in pure powdered form and have no added preservative.

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NA

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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