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Research Article

EVALUATION OF SOME HERBAL SOLID DOSAGE FORMS FOR MICROBIAL CONTAMINATION

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ABSTRACT

In the treatment and prevention of various diseases, herbal formulations are widely used and often contain high pharmacological active compounds. These herbal products have the potential of contamination with different microorganisms. Contamination of herbal products with microorganisms could make changes in physico-chemical characteristics as well as the toxicity of pharmaceutical preparations. All the contents of the dosage forms (active ingredients and excipients) are susceptible to microbial contamination and spoilage. It occurs during production like raw material, unhygienic environmental and production conditions. In this study, microbiological quality of some herbal solid dosage forms from public markets, in the city of Meerut, India was examined. 20 herbal products as tablet, powder and capsule were evaluated for microbial contamination by USP (United States Pharmacopoeia) microbial limit test for enumeration and identification. Total aerobic count showed that all products had more than 1100 microorganism per gram. Isolation and identification of microbial contamination showed that all the samples were contaminated with *Salmonella* sp. and there was no evidence for contamination of the samples by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. In conclusion, all the samples of herbal drugs evaluated did not generally meet the standards for microbial limits as specified in official monographs. Such products can adversely affect health status of consumers as well as the stability of the products.

Keywords: Microorganism, Herbal, Contamination, Solid dosage form.

INTRODUCTION

Medicinal plants have a long history of use in therapy throughout the world and still make an important part of traditional medicine. Thus, medicinal plants and herbal products must be safe for the patient (consumer).[1] 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.[2] 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.[3] The microbial quality of pharmaceuticals is influenced by the environment and quality of the raw materials used during formulation. Some infections outbreaks have been associated with the use of heavily contaminated raw materials of natural origin. The incidence of micro flora in non-sterile medicines generally is indicated by the nature of the ingredients (whether natural or synthetic), the quality of the vehicle, the care and attitude of persons involved in their handling among others. 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 tablets 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[4]. 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. Therefore it is necessary to estimate the microbial contamination level on crude drugs at each manufacturing stage, reported the bacterial contamination of some herbal solid dosage forms. In their research, herbal powders were contaminated with *Salmonella* and *Escherichia coli* and herbal tablets were contaminated with *E. coli* [5]. Limyati and Juniar (1998) conducted an examination on the microbiological quality of seven kinds of Jamu Gendong (a kind of traditional medicine in liquid or other form that is freshly prepared from plant material) and their raw materials[6]. They concluded that in most cases, the Jamu Gendong samples were heavily contaminated with bacteria. Govender et al (2006) assessed the microbial quality of herbal medicines from shops in the Nelson Mandela Metropolis. They found significant contamination by bacteria and fungi.[7] Contamination of 84 medicinal plant samples and spices by fungi and their mycotoxins were examined. The pharmaceutical and microbial qualities of 21 different brands of herbal medicinal products in Southwestern Nigeria were evaluated. The microbial load of the products varied considerably 47.6% of the samples were contaminated by *E. coli*, 33% were contaminated by *Salmonella*, 71.4% were contaminated by *Staphylococcus aureus* and 57.1% were contaminated by fungi.[8] Aziz et al (1998) were evaluate the possible microbial contamination of 20 herbal solid dosage forms. Ten fungal genera of different taxonomic groups were detected .[9]

MATERALS AND METHODS

Materials

All cultures media (soybean casein digest agar medium, fluid soybean-casein digest medium, sabouraud dextrose broth, manitol-salt agar medium, Vogel-Johnson agar medium, fluid lactose medium, cetrimide agar medium, mac-conkey agar medium, selenitecystine medium, bluid tetrationate medium, brilliant green agar medium, bismuth sulfite agar medium, triple sugar-iron agar medium, sabouraud dextrose agar) and chemicals (potassium tellurite, glycerin brilliant green, potassium iodide, iodine) were obtained from Sigma-Aldrich Corporation Bangalore, India.

Microorganisms

The indicator microorganisms used in this study were all collected from the National Collection of Industrial Microorganism (NCIM) Pune, India and included: *S. aureus*, *E. coli*, *Pseudomonas aeruginosa, Salmonella typhi* and *Candida albicans.*

Preparation of samples

Twenty different herbal solid dosage forms were collected at random from markets, in the city of Meerut, India. The type of dosage form, packaging, manufacturing dates and expiry dates are presented in Table 1. 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 equipments.[10]

Inoculation of microorganisms for recovery study

1 ml of not less than 10^{-3} dilution of a 24-h broth culture of the indicator micro- 0rganisms (*E. coli, P. aeruginosa, S. aureus,* S. *typhi*

and *C. albicans* were added to the solid dosage form samples (in fluid soybean-casein digest medium or sabouraud dextrose broth), then incubated for 48 - 72 h and were evaluated for microbial growth in comparison with the colony morphology of positive blank (culture medium plus related microorganism). Doubtful results were confirmed by subculturing on selective media .[11]

Table 1: The type of dosage form, packaging, manufacturing and expiration dates of subject solid herbal drugs. (These herbal dosage forms were use as dispensed.)

Product code	Dosage form	Packaging	Manufacturing Country	Manufacturing date	Expiration date
A	Tablet	Blister	INDIA	10/2007	10/2010
B	Tablet	Blister	INDIA	09/2008	09/2011
C	Tablet	Blister	INDIA	7/2008	7/2011
D	Tablet	Blister	INDIA	4/2006	4/2010
E	Tablet	Blister	INDIA	7/2006	7/2010
F	Tablet	Blister	INDIA	11/2007	10/2010
G	Tablet	Blister	INDIA	1/2007	1/2010
H	Tablet	Bulk	INDIA	12/2007	12/2010
I	Tablet	Bulk	INDIA	8/2008	7/2011
J	Tablet	Bulk	INDIA	1/2007	12/2010
K	Tablet	Bulk	INDIA	3/2008	7/2011
L	Tablet	Bulk	INDIA	3/2007	2/2010
M	Powder	Bulk	INDIA	6/2007	5/2010
N	Powder	Bulk	INDIA	4/2007	4/2010
O	Powder	Sachet	INDIA	4/2008	4/2011
P	Powder	Blister	INDIA	10/2007	11/2010
Q	Capsule	Blister	INDIA	7/2006	12/2009
R	Capsule	Blister	INDIA	6/2007	6/2010
S	Capsule	Blister	INDIA	5/2008	5/2011
T	Capsule	Blister	INDIA	2/2007	1/2010

Media and isolation of pathogenic microorganisms

To determine the presence of *S. aureus* and *P. aeruginosa*, each sample diluted to 100 ml by adding soybean-casein digest medium and then incubated. After growth, a portion of the medium was spread on the surface of Vogel-Johnson agar and manitol-salt agar for detection of *S. aureus* and of cetrimide agar medium for detection of *P. aeruginosa*. Fluid lactose medium was added to 10 g of each sample to make 100 ml for detecting *E. coli and Salmonella* sp. Fluid lactose enrichment were streaked onto differential Mac-Conkey agar plates, while 1ml aliquots of the fluid lactose cultures were transferred into 9ml fluid selenite-cystine and fluid tetrationate, respectively, to detect *Salmonella* sp. These cultures were incubated at 35 ± 2°C for 12 to 24 h and were further sub cultured on the surface of brilliant green agar and bismuth sulfite agar media. The butt-slant tube of triple sugar-iron agar medium was used for identification of gram-negative rods colonies. On the other hand, 10 g of each sample were added to sabouraud dextrose broth to make 100 ml for detection of *C. albicans*. Sabouraud dextrose broth enrichments were incubated at 20 - 25°C for 7 days. The incubated samples were examined and cultured in sabouraud dextrose agar plus chloramphenicol (SDA + C). In cases where microbial growth was observed, the colonies were identified by germ-tube test and morphological characteristics were examined microscopically.[10]

Bioburden determination

The collected samples of herbal products were subjected to the following examinations: total aerobic viable count (TAVC) 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. 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 USP 30 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.[10]

Table 2: Identification, isolation and microbial count of some herbal solid dosage forms

Product code	Mean plate counts (cfu.g-1)	Multiple tube counts (cfu.g-1)	Isolated organism(s)
A	5.2 × 104	> 1050	*Salmonella* sp.
B	6.5 × 104	> 1050	*Salmonella* sp.
C	4.2 × 104	> 1050	*Salmonella* sp.
D	3.5 × 104	> 1050	*Salmonella* sp.
E	1.2 × 104	> 1050	*Salmonella* sp.
F	5.5 × 104	> 1050	*Salmonella* sp.
G	5.1 × 104	> 1050	*Salmonella* sp.
H	6.5 × 104	> 1050	*Salmonella* sp.
I	5.0 × 104	> 1050	*Salmonella* sp.
J	7.5 × 104	> 1050	*Salmonella* sp.
K	10.0 × 104	> 1050	*Salmonella* sp.
L	3.4 × 104	> 1050	*Salmonella* sp.
M	7.9 × 104	> 1050	*Salmonella* sp.
N	4.3 × 104	> 1050	*Salmonella* sp.
O	7.4 × 104	> 1050	*Salmonella* sp.
P	8.2 × 104	> 1050	*Salmonella* sp.
Q	9.7 × 104	> 1050	*Salmonella* sp.
R	6.0 × 104	> 1050	*Salmonella* sp.
S	10.3 × 104	> 1050	*Salmonella* sp.
T	7.5 × 104	> 1050	*Salmonella* sp.

RESULTS

The results of preparatory testing (data not shown) indicated that all used specimens do not inhibit the multiplication of indicator microorganisms under the test conditions. The microbial levels of herbal solid dosage forms used in this study as depicted in Table 2 showed that all of the samples had microbial contaminants. Microbial counts by plate method ranged between 1.4×10^4 and 10×10^4 cfu/g and by multiple tube method was >1050 cfu/g in all samples. The presence of indicator organisms in the samples is reported in Table 2. 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. The presence of *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans* were not observed in any of the 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.

DISCUSSION

Based on US pharmacopoeia (USP 30), the total aerobic microbial count of dried or powdered botanical ingredients and products was not more than 10^5cfu/g. Thus, the microbial levels of solid dosage forms used in this study were acceptable except for the 11 code. The type of herbal solid dosage forms packaging that were blister for some tablets and capsules and bulk for some tablets. Powders probably do not have any effect in microbial counts of samples. This finding demonstrated that raw materials of natural origin in these formulations had some initial microbial levels of contaminants which related to the growing and culture conditions of medicinal plants. Similar findings have also been obtained in an earlier study on the microbiological quality of some pharmaceutical raw materials (Westwood, 1971)[12]. Same results have also been obtained in an earlier study on the microbiological quality of some herbal products (R. Enayatifard 2009)[13]. The microbial levels associated with these herbal dosage forms could be attributed to their source of origin and their nutritive values and low standard of processing. On the other hand, high total plate counts do not have any correlation with the presence of pathogenic microorganisms. The presence of bacteria in herbal solid dosage forms constitutes a health hazard, particularly with Salmonella species which are the causative agents of harmful diseases. The presence of these harmful bacteria might be due to the application of manure to fertilize farms from which medicinal plants have been harvested. Animal manure and slurries may contain a wide range of pathogenic microorganisms such as salmonella species. These organisms may survive for extended periods of time in soil and thus, increase the risk of plant contamination. Moreover, in the absence of viable cells, microbial metabolites may be toxic (Baird, 1992; Beveridge, 1992)[14,15]. Similar results were obtained with the herbal solid dosage forms of the powder samples contaminated by *E. coli* and *Salmonella* species and herbal tablets contaminated by *E. coli* (Bahri Najafi et al., 2001)[5]. According to WHO report (2002), *Salmonella* food poisoning is a major problem globally and has increased in incidence in many continents in the last 25 years. *Salmonella* can infect plants cells and successfully evade all the defense mechanisms of plants. This shows that cleaning the surfaces of raw fruits and vegetables, for example, by washing, are not sufficient to protect against food poisoning. Previously, the only known sources of infection were plants contact with contaminated water (Goyal et al., 1977; Kudva et al., 1998; Solomon et al., 2002)[16,11,17]. But recent studies showed that the strain of bacteria known as *S. typhimurium* can also invade and multiply inside plant cells. It is already known that *Salmonella* can survive for up to 900 days in contaminated soils, which creates a rich source of infection for plant material. However with this study, the hazard of microbial contamination of herbal solid dosage forms during manufacturing to human health has been demonstrated.

CONCLUSION

On the basis of the results, the microbiological quality of some herbal solid dosage forms are influenced to varying degrees by the microbial levels of the starting raw materials, probably the production method and the production environment. Moreover, high counts of harmful microorganisms such as *Salmonella* species may affect the human health and drug quality and these emphasizes the necessity of improving plant material quality and establishing better hygienic conditions of herbal solid dosage forms production. Quality has to be built into the whole process beginning from the selection of propagation material to the final product reaching the consumer. Thus, there is need for constant monitoring and control of the standards of herbal medicines available in the market.

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