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Full Length Research Paper

Microbial quality of some herbal solid dosage forms

R. Enayatifard, H. Asgarirad* and B. Kazemi-sani

Department of Pharmaceutics, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

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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. In this study, microbiological quality of some herbal solid dosage forms from public markets, in the city of Sari, Iran was examined. 20 herbal products as tablet, powder and capsule were prepared. The products 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.

Key words: Microbial quality, herbal, contamination, solid dosage form

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 (Hitokoto et al., 1978). 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 (Nikajima et al., 2005). 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 (Akerele and Godwin, 2002). 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.

Bahri et al. (2001) 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* (Bahri Najafi et al., 2001). 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

*Corresponding author. E-mail: dr_asgarirad@yahoo.de. Tel: +98-151-3243082. Fax: +98-151-3243084.

Table 1. The type of dosage form, packaging, manufacturing and expiration dates of subject solid herbal drugs.

Product code	Dosage form	Packaging	Manufacturing date	Expiration date
1	Tablet	Blister	12/2005	12/2008
2	Tablet	Blister	11/2005	11/2009
3	Tablet	Blister	9/2007	9/2010
4	Tablet	Blister	6/2005	8/2009
5	Tablet	Blister	9/2005	7/2009
6	Tablet	Blister	12/2005	11/2008
7	Tablet	Blister	2/2006	2/2009
8	Tablet	Bulk	1/2006	1/2010
9	Tablet	Bulk	10/2005	9/2009
10	Tablet	Bulk	2/2005	1/2009
11	Tablet	Bulk	4/2006	8/2009
12	Powder	Bulk	4/2005	3/2008
13	Powder	Bulk	7/2005	6/2008
14	Powder	Bulk	3/2006	3/2009
15	Powder	Sachet	5/2007	5/2010
16	Capsule	Blister	11/2006	12/2009
17	Capsule	Blister	8/2004	1/2008
18	Capsule	Blister	7/2006	7/2009
19	Capsule	Blister	5/2005	5/2008
20	Capsule	Blister	2/2005	1/2008

These herbal dosage forms were use as dispensed.

prepared from plant material) and their raw materials. 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. Contamination of 84 medicinal plant samples and spices by fungi and their mycotoxins were examined. Ten fungal genera of different taxonomic groups were detected (Aziz et al., 1998). The pharmaceutical and microbial qualities of 21 different brands of herbal medicinal products in Southwestern Nigeria was 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 (Okunlola et al., 2007). The aim of this study was to evaluate the possible microbial contamination of 20 herbal solid dosage forms.

MATERALS 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, selenite-cystine 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, potassium iodide, iodine, brilliant green) were obtained from Merck chemical co. (Darmstadt, Germany).

Microorganisms

The indicator microorganisms used in this study were all from the Persian Type Culture Collection (PTCC) and included: *S. aureus* PTCC 1112 (ATTC 6538), *E. coli* PTCC 1330 (ATTC 8739), *Pseudomonas aeruginosa* PTCC 1074 (ATCC 9027), *Salmonella typhi* PTCC 1639 (ATCC 19430) and *Candida albicans* PTCC 5027 (ATCC 10231).

Preparation of samples

Twenty different herbal solid dosage forms were collected at random from public markets, in the city of Sari, Iran. The type of dosage form, packaging and manufacturing 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, (USP 30, 2007).

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-organisms (*S. aureus* PTCC 1112, *E. coli* PTCC 1330, *P. aeruginosa* PTCC 1074, *S. typhi* PTCC 1639 and *C. albicans* PTCC 5027) 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 (Kudva et al, 1998).

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.

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.

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 1.5×10^4 and 11×10^4 cfu/g and by multiple tube method was >1100 cfu/g^1 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). 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). Similar results were obtained with the herbal solid dosage forms of the powder samples

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)
1	6.3×10^4	> 1100	*Salmonella* sp.
2	7.5×10^4	> 1100	*Salmonella* sp.
3	3.2×10^4	> 1100	*Salmonella* sp.
4	1.5×10^4	> 1100	*Salmonella* sp.
5	10.2×10^4	> 1100	*Salmonella* sp.
6	2.5×10^4	> 1100	*Salmonella* sp.
7	5.4×10^4	> 1100	*Salmonella* sp.
8	6.5×10^4	> 1100	*Salmonella* sp.
9	5.0×10^4	> 1100	*Salmonella* sp.
10	7.05×10^4	> 1100	*Salmonella* sp.
11	11.0×10^4	> 1100	*Salmonella* sp.
12	6.4×10^4	> 1100	*Salmonella* sp.
13	4.9×10^4	> 1100	*Salmonella* sp.
14	7.3×10^4	> 1100	*Salmonella* sp.
15	8.8×10^4	> 1100	*Salmonella* sp.
16	9.2×10^4	> 1100	*Salmonella* sp.
17	7.7×10^4	> 1100	*Salmonella* sp.
18	7.0×10^4	> 1100	*Salmonella* sp.
19	10.1×10^4	> 1100	*Salmonella* sp.
20	7.7×10^4	> 1100	*Salmonella* sp.

contaminated by *E. coli* and *Salmonella* species and herbal tablets contaminated by *E. coli* (Bahri Najafi et al., 2001). 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). 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.

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