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Microbial contamination of locally available medicinal herbs in Mansehra, Pakistan

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Abstract

Microbial contamination of herbal materials can cause the spoilage of pharmaceuticals to a great extent. Evaluation of microbial loads of plant materials to assure quality is therefore significant investigation. In the present study, eight commercially available medicinal plants were evaluated for bacterial and fungal contamination. The results of present study revealed very high microbial loads and also the presence of some pathogenic bacteria in herbal materials. The bacterial strains identified in the investigation were *Staphylococcus epidermydis, E.coli, Pseudomonas, Enterobactar aerogenosa, Staphylococcus aureus and Klebsiella*. The fungal isolates were *Rhizopus oryzae, Aspergillus Niger, Aspergillus flavus, Aspergillus fumigates, Cladosporium herbarum, Mucor hiemalis and Penicillium chrysogenum*. It was concluded that commercially available plants in Mansehra may be at high risk of microbial contamination and not suitable for medicinal use.

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Introduction

The future of the plant-based health products and industries is enormously strong as the recent social and cultural tendencies toward natural healing and healthy diets are increasingly growing. Scientists all over the world are therefore trying to explore the precious assets of medicinal plants to help the suffering humanity (Hussain *et al.*, 2009). The number of reports of patients experiencing negative health impacts investigated by the use of herbal materials has also been rising globally (Banarjee and Sarkar, 2003). One of the major reasons of reported detrimental actions is directly associated with the poor quality of plant materials. Generally, plant materials are contaminated with high levels of bacteria, molds, and yeasts.

The presence sufficient numbers of microbes can be harmful to consumers. As a result of fungal contamination, the risk of mycotoxin production, especially aflatoxin, should be taken into consideration in the manufacturing process because of the proven mutagenic, carcinogenic, teratogenic, neurotoxic, nephrotoxic, immunosuppressive activities (Refai, 1988; Scimeca, 1995; FAO, 2000; Hohler, 2000). The microbial quality of herbal drugs has to be co-coordinated with the regulations of the European Pharmacopoeia 6th Edition and Regulations of medical safety of dietary ingredients. Despite several reports of fungal contamination and aflatoxin production in foodstuff, limited research has been carried out on the microbial contamination of drug-plant samples.

The microorganisms found in plants are usually native to the soils and surroundings in which the plants are grown. A broad range of microorganisms and microbial loads has been reported in medicinal plants earlier (Lutomski & Kedzia, 1980; Baxter & Holzapfel, 1982; Kneifel & Berger, 1994; Czech *et al.*, 2001; Garcia *et al.*, 2001). The presence of microbial contaminants in plant materials may affect the usefulness and stability of the active compounds and for that reason reduces or inactivates the medicinal activity. These contaminants therefore have a great potential to adversely affect the health of patients. Pathogenic microorganisms may also grow on some herbs. The consumers may possibly fall ill because of taking herbs incriminated with pathogenic microorganisms. The contaminated materials can also cause the spoilage of conventional herbal and pharmaceutical preparations, to which they are added.

A few authors have indicated microbial contamination of medicinal plants from various parts of the world. Halt (1998) isolated a wide spectrum of fungi including *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Rhizopus* and *Mucor* species from Croatian herbal teas and medicinal plants. He also found that most of the fungal species found in the organo herb (*Origanum vulgare* L.) were from the genus *Aspergillus*, and less from the genus *Alternaria*, *Rhizopus* and *Penicillium*. Examination of the microbial quality of mint has shown that the most abundant fungi were from *Penicillium, Alternaria* and *Fusarium* according to Stojadinov J. (1998), or *Fusarium* and *Verticillium* (Pavlović *et al.*, 2000; Stević *et al.*, 2004), as well as *Alternaria alternata, Aspergillus flavus, A. ochraceus, Penicillium cyclopium, Fusarium culmorum, F. equiseti, F. semitectum* and *Septoria menthae*. A perusal of different reports on fungal and mycotoxin contamination of raw materials indicates that there is no uniformity in the association of fungal species with raw materials. This may be because of the presence of specific secondary metabolites in different herbal drugs, which may be fungitoxic in nature for some fungal species and provide chemical resistance against them (Dubey *et al.*, 2008).

The business of medicinal plants remains active throughout the year in the country. But unfortunately, no logical attempt has yet been made for systematic exploitation and industrial utilization of these valuable natural resources. The production and standardization of traditional medicines is still not very well coordinated in Pakistan. Traditional

medicines are widely distributed and used without former testing for safety and microbial quality. Assessment of microbiological status of plant materials to declare safety and quality is worth investigation. The present study was designed to throw light on the microbial quality of commercially available herbal materials in the city of Mansehra. The objective of this investigation was to determine the microbial quality (presence of bacteria and fungi) of the herbal drugs that are mostly used in the manufacture of various herbal medicines.

Materials and methods

Collection of Medicinal Herbs

Whole plant or parts of different plants were purchased from the local market of Mansehra. The plant materials were collected aseptically with gloves into sterile polyethylene pouches. During this investigation eight sampling materials were collected from different retail shops located in different areas of Mansehra city. These materials were including different parts of medicinal herbs. These herbs were including Anab, Sanghara, Bhery, Mamekh, Jaman, Tulsi, Taramira and Ajwain. All the collected medicinal herbs were examined physically under stereomicroscope and were found contaminated with dust, soil and different colored stains. It has also been observed that these medicinal herbs were also not kept in proper jars which might be a source of contamination. A detail of collected medicinal herbs, botanical names and the traditional use is given in table 1.

Table 1. Collected medicinal herbs, botanical names and the traditional uses.

Serial number	Local names	Botanical names	Traditional uses
1	Anab	*Zizyphus sativa*	Used for nerves weakness, diarrhea, sore throat, malaria, chronic fever, eczema and chest complaints
2	Sanghara	*Trapa bispinosa*	Used as a blood tonic, bitter and astringent
3	Bhery	*Terminalia belerica*	Used as a carminative, mouth freshener, and for antimicrobial action.
4	Mamekh	*Paeonia emodi*	Used for fever, chest disease and chronic diarrhea
5	Jaman	*Eugenia jambolana*	Used for Jaundice. Seed's oil is used for ulcer and against itching.
6	Tulsi	*Ocimum sanctum*	Used as an insect repellent and to treat common cold, stomach disorders and malaria
7	Taramira	*Eruca sativa*	Oil is used for massage and skin infections
8	Ajwain	*Trachyspermum copticum*	Used for gastric troubles

Sample Preparation

Plant samples were cleaned to remove adhering soils and other unwanted extraneous materials. The dried plant materials were then ground individually in a grinder (Retch Muhle-Germany) so that the whole of the material passed through a 30 mesh sieve. The powdered samples were packed (150 gm each) in clear polyethylene pouches, sealed with an electric sealer and stored at 4°C until further analysis.

Isolation of Microbes

All microbiological analyses were carried out in triplicate. The total bacteria in plant samples were determined by the surface plate agar method with a medium containing nutrient agar medium. Each suspension was properly diluted with the same sterile water, as material being examined. Aliquots of 0.2 ml from each dilution were then spread on the surface of the agar plates. The bacteria were counted after 3 days incubation at 30°C. The specific bacterial species were identified by different biochemical tests including catalase, coagulase, Oxidase, citrate test, indole, VP test and methyl red test.

For fungal species each contaminated part of the plant was directly put on freshly prepared Potato dextrose agar. Fungal count of the plant materials was determined by plate count method using potato dextrose agar. Plates were incubated at a temperature of 28°C for 3-5 days and the number of colony-forming units (cfu) was counted.

Results

Fungal Contamination of Medicinal Herbs

During present investigation 7 fungal species were isolated from contaminated parts of medicinal herbs collected from different areas of Mansehra city. These fungal species were included *Rhizopus oryzae , Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Cladosporium herbarum, Mucor hiemalis* and *Penicillium chrysogenum*. The detail of these fungal species along with medicinal herbs is given in table 2. The fungal species *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Cladosporium herbarum* were found prominent fungal species isolated from collected materials.

Table 2. Fungal species isolated from contaminated medicinal herbs.

Serial number	Botanical names	Number of colonies	Fungal species
1	*Zizyphus sativa*	5	*Rhizopus oryzae, Aspergillus niger, Aspergillus flavus*
2	*Trapa bispinosa*	1	*Cladosporium herbarum*
3	*Terminalia belerica*	4	*Cladosporium herbarum , Aspergillus fumigates, Aspergillus niger*
4	*Paeonia emodi*	5	*Aspergillus fumigates, Aspergillus niger*
5	*Eugenia jambolana*	6	*Cladosporium herbarum*
6	*Ocimum sanctum*	4	*Aspergillus niger*
7	*Eruca sativa*	2	*Mucor hiemalis, Aspergillus flavus*
8	*Trachyspermum copticum*	6	*Rhizopus oryzae, Aspergillus flavus Penicillium chrysogenum*

Bacterial Contamination of Medicinal Herbs

During present investigation 6 bacterial species were isolated from contaminated parts of medicinal herbs collected from different areas of Mansehra city. These bacterial species were included *Staphylococcus epidermydis, E.coli, Pseudomonas, Enterobacter aerogens, staphylococcus aurus* and *Klebsiella*. The detail of these bacterial species along with medicinal herbs is given in table 3. The bacterial species *Staphylococcus epidermydis, E.coli,* and *Enterobacter aerogens* were found prominent bacterial species isolated from collected materials.

Table 3. Bacterial species isolated from contaminated medicinal herbs.

Serial number	Local Names	Botanical Names	Bacterial Species Identified
1	Anab	*Zizyphus sativa*	*Staphylococcus epidermydis, E.coli, Pseudomonas*
2	Sanghara	*Trapa bispinosa*	*Staphylococcus epidermydis, E.coli, Pseudomonas*
3	Bhery	*Terminalia belerica*	*Staphylococcus epidermydis, Enterobacter aerogens, Klebsiella*
4	Mamekh	*Paeonia emodi*	*Streptococcus spp., Enterobacter aerogens, Pseudomonas*
5	Jaman	*Engenia jambolana*	*Staphylococcus aureus, Enterobacter aerogens, Klebsiella*
6	Tulsi	*Ocimum sanctum*	*E.coli , Pseudomonas, Staph.epidermydis*
7	Taramira	*Eruca sativa*	*Enterobacter aerogens, Klebsiella, Staphylococcus. epidermydis*
8	Ajwain	*Trachyspermum copticum*	*E.coli, Enterobacter aerogens, Pseudomonas, Staphy lococcus. aureus*

The fungal count in selected plant materials are similar to those reported previously by Czech *et al.* (2001), Phianphak *et al.*, (2007) and Idu *et al.* (2008). None of the sample showed fungal count under permissible levels. Previously, Khan *et al.* (2006) reported the presence of *Fusarium oxysporum, Alternaria* spp., *Penicillium* spp., *Aspergillus niger* and *Botrytis cinerea* in stored samples of medicinal plants.

The present study revealed that commercially available plants might be high-risk materials, as it contained very high microbial load and pathogenic microorganisms. The contaminated materials may

cause spoilage and other quality defects in herbal and pharmaceutical preparations. When impregnated with pathogenic bacteria, they can cause serious illnesses.

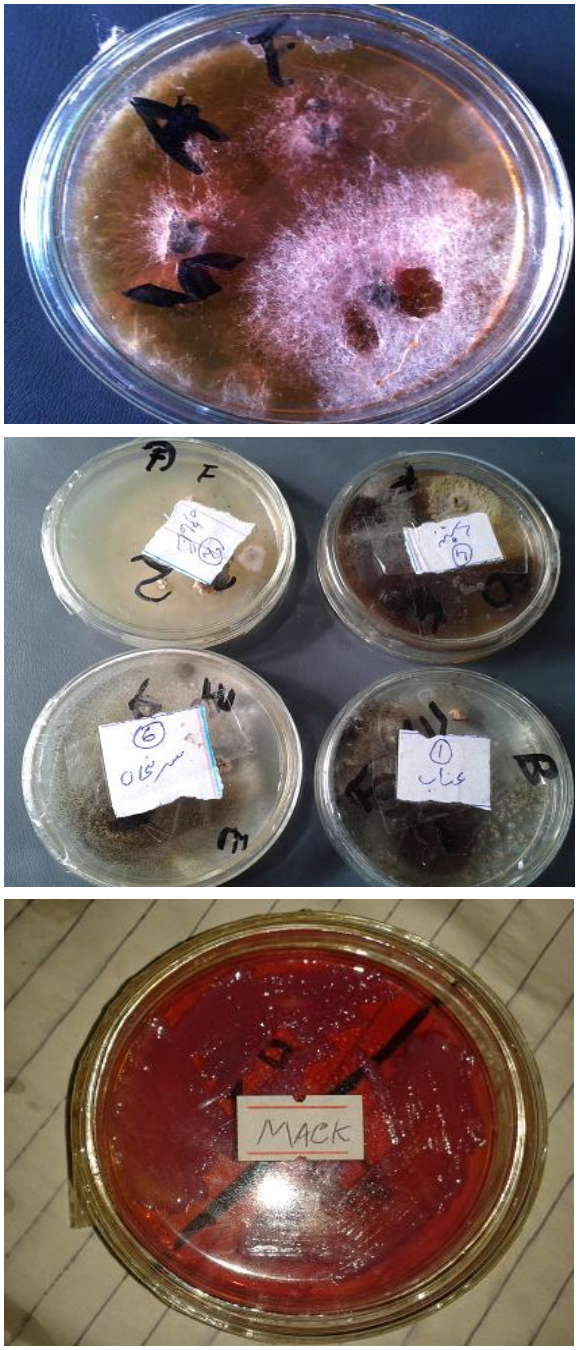

Fig. 1-3. Growth of fungal and bacterial species on selective media.

In Pakistan, major portions of herbal materials are collected from wild areas that may be highly contaminated by dust, soil, air borne spores. In present investigation During 7 fungal species were isolated from contaminated parts of medicinal herbs collected from different areas of Mansehra city. These fungal species were included *Rhizopus oryzae , Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Cladosporium herbarum, Mucor hiemalis* and *Penicillium chrysogenum*. While in present investigation 5 bacterial species were isolated from contaminated parts of medicinal herbs collected from different areas of Mansehra city. These bacterial species were included *Staphylococcus epidermydis, E.coli, Pseudomonas, Enterobacter aerogens* and *Klebsiella* Similar results have earlier been reported by Abou-Donia (2008). The highest mean count of *E. coli* was detected in *Ficus glomerata*. The presence of *E. coli* shows the increased risk for plant-borne illness.

Earlier research studies showed different results for *Salmonella* counts in plant materials. A study conducted by Phianphak *et al*. (2007) showed high counts for *Salmonella* in herbal materials, while Abou-Donia (2008) reported that Egyptian spices and medicinal plants are free of *Salmonella* spp. Almost all serotypes of *Salmonella* are pathogens for humans, which is a serious health concern (Lee and Jo, 2006). The plant materials may be contaminated with *Salmonella* by human handlers during harvesting or processing. Fungi are extensively found in the atmosphere and they have been recognized as the quality criteria for products exposed to open air. All the samples included in the study were contaminated with fungi, as shown by total fungal counts, which is in the range of 2.8 x 102 to 8.6 x 106 cfu/g.

Therefore, there is urgent need to frequently check the quality of medicinal plants that are on sale in the open market in order to keep a suitable standard for plant materials destined for human consumption. Considering the severe health risks, processing methods such as harvesting, drying, transportation and storage must also be improved and WHO guidelines on good agricultural and collection

practices (GACP) ought to be followed strictly. More studies are also needed to categorize the risk factors for the presence of high load of microorganisms and check the impact of herbs contamination on public health. There is a need of screening of traditional knowledge through scientific evidences as recently being carried out.

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