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3MICROBIOLOGICAL QUALITY ASSESSMENT OF COMMERCIALLY AVAILABLE MEDICINAL PLANTS IN PESHAWAR CITY, PAKISTAN

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Abstract

Medicinal plants naturally harbor a variety of microorganisms. Besides, representing a direct health hazard to the consumer, these contaminated materials can cause the spoilage of pharmaceuticals and traditional preparations to which they are added. Assessment of microbiological loads of plants to assure safety and quality is therefore worth investigation. In the present study, 45 commercially available medicinal plants were evaluated for aerobic bacteria, fungi, coliforms, *E. coli* and *Salmonella*. All investigations were carried out in triplicate using standard methods. The results of the study revealed very high microbial loads and the presence of pathogenic bacteria in the plant samples. The aerobic bacterial count ranged from1.3 x 10^2 to 5.6 x 10^9 cfu/g. The highest load was detected in the rhizomes of *Curcuma longa*. The coliform counts varied from 1.5 x 10^2 to 1.6 x 10^4 cfu/g. Among the selected herbs, 23 showed the presence of *E. coli*, while *Salmonella* spp. was detected in 13 samples. The fungal counts were above the international permissible level in the tested samples. It was concluded that commercially available plants may be high-risk substances and therefore quality of the plants may be regularly checked to ensure safety and make them fit for human consumption.

Introduction

Treatment using medicinal plants started since the beginning of the history. The ancient people strongly believed in the healing effects of plants and extensively used these for various health disorders (Shinwari, 2010). Historically, the nations like Assyrians, the Persians, the Babylonians, the Greeks, the Chinese, the Indians, and the Egyptians have a rich account of using medicinal plants. The bulk of the population in many developing as well as developed countries still use traditional medicines, which have not been fully discovered or appreciated in modern science-based therapy (Said, 1996; Eisenberg *et al.*, 1998). According to an estimation of WHO, about 80% of world population rely on medicinal plants for their basic health care needs, and more than 30% of the pharmaceutical preparations are based on the active ingredients of plants (Akerele, 1993; Shinwari & Gilani, 2003; Anon., 1998).

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*., 2009a). WHO has declared traditional medicine as one of the efficient means to achieve the total health care coverage of the world's population. WHO has developed inclusive technical guidelines for the appraisal of herbal medicine and totally supported the rational use of traditional plants based medicines by its member states (Anon., 1998; Anon., 2000). However, the number of reports of patients experiencing negative health impacts instigated by the use of herbal materials has also been rising globally (Anon., 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, moulds, and yeasts. 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; Kneifel *et al.*, 2002; Banarjee & Sarkar, 2003). 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. According to international requirement (Anon., 1984), the total count of aerobic bacteria in herbal raw materials should not exceed 10000 in 1g or 1ml. Similarly, fungi should not be higher than 100 in 1g or 1 ml and *Escherichia coli* (*E. coli*) must be absent.

There are around 6000 types of wild plants in Pakistan, out of which about 700 are considered medicinally important (Shinwari & Qaiser, 2011). These plants are extensively used for the treatment of various health problems in the country (Saeed & Rizvi, 1990; Nasir & Rafiq, 1995; Said, 1996; Islam *et al.*, 2006; Ahmad, 2007; Hussain *et al.*, 2008, 2009). A large number of tabibs (practitioners of Greco-Arab medicine) and a number of unregistered practitioners in urban, rural and remote hilly areas are utilizing these plant materials in traditional and folk recipes. The dried plant materials are also available in the local markets. Consumers directly purchase and use these herbal materials in traditional styles to make infusions. Typically, the powdered or crushed parts of the plants are ingested with drinking water as household remedies, without any further treatment.

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 Peshawar.

Materials and Methods

Materials: Whole plants or parts of different plants (Table 1) were purchased form the local market of Peshawar, Pakistan. The plant materials were collected aseptically with gloves into sterile polyethylene pouches.

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.

Enumeration of microbial load: All microbiological analyses were carried out in triplicate. The total aerobic mesophilic bacteria in plant samples were determined by the surface plate agar method with a medium containing Difco-nutrient agar 23 g, glucose 5 g, yeast extract 5 g, and dipotassium phosphate 2 gm per liter (pH 7.0). Two gram of each plant sample was added to 20 ml of 0.01% Tween-20 sterile water and mixed thoroughly. Each suspension was properly diluted with the same sterile water, as necessary depending on the expected bacterial load of the 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 total aerobic mesophilic counts were calculated by multiplying the average number of colonies with dilution factor and reported as a colony-forming unit per gram (cfu/g) of sample. The coliform bacteria were determined using MacConkey agar media for 24 hours incubation at 37°C. Pink colonies were counted and calculations were made. Presumptive *E. coli,* colonies appearing on MacConkey agar media were then subcultured on eosin methylene blue agar (EMB) and incubated at 37°C for 24-48 hours. Colonies with characteristic greenish metallic color were subjected to IMVIC tests to identify *E. coli*. For *Salmonella* spp., 25 g of sample was aseptically weighted and added into 225 ml of sterile lactose broth and incubated at 37°C for 18-20 h. An inoculum of 0.2 ml was transferred into a tube containing 9.8 ml of selenite cystine broth by subsequent incubation at 40°C for 24 hours and afterward plated onto bismuth sulphite agar and xylose lysine desoxycholate agar plates for 24-48 hours at 37°C and then examined for typical colonies. 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) were counted.

Results and Discussion

The microbiological quality of medicinal plants obtained from the local market of Peshawar is shown in Table 2. The plant samples showed elevated levels of contamination and were contaminated to varying degree with bacteria and fungi. The counts of total aerobic bacteria in plant materials ranged from 1.3×10^2 to 5.6×10^9 cfu/g. This is in agreement with the higher levels of aerobic bacteria found in other herbal materials, reported earlier by several researchers (Czech *et al.*, 2001; Phianphak *et al.*, 2007; Abou-Donia, 2008). While in contrast to the findings of the present study, Idu *et al*. (2008) discovered comparatively low bacterial counts (varied from 10^2 to 10^4 cfu/g) in medicinal plant samples. This difference in the total count of aerobic bacteria may be due to the variation in plant's types, geographical distributions, environmental factors, processing techniques and storage conditions etc. The rhizomes of *Curcuma longa* showed the highest aerobic count. Fourteen plant samples showed the counts of aerobic bacteria under permissible levels (Anon., 1984) for herbal raw materials. Generally, plate count of aerobic bacteria in herbs is considered as a sign of general sanitation and quality parameter (Kneifel *et al*., 2002). High bacterial load found in the samples may reflect that plants had been exposed to poor handling, unsuitable processing methods and inapt storage condition. Coliforms represent one of the most important bacterial groups with reference to safety and quality of biological materials. In the present investigation, the coliform bacteria were detected in 23 herbs and the counts ranged from 1.5×10^2 to 1.6×10^4 cfu/g. The highest mean count was noted for *Cyperus rotundus*. Previously, a number of researchers (Czech *et al.*, 2001; Phianphak *et al.*, 2007; Idu *et al*., 2008) reported high coliforms counts ranging from 10^2 to 10^7 in herbal materials. Whereas, a study conducted by Abou-Donia (2008) showed a comparatively low coliforms count (10^1 to 10^3) in Egyptian spices and medicinal plants. The occurrence of coliforms show the possibility of fecal contamination and inadequate sanitation conditions while the plants were being grown. In Pakistan, major portions of herbal materials are collected from wild areas that may be highly contaminated by the feces of wild animals. *E. coli* was detected in 23 plants and the count ranged from 1.4×10^1 to 6.3×10^3 cfu/g. 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. The results of the study showed the prevalence of *Salmonella* spp., in 13 plant samples. The colony-forming unit for *Salmonella* spp. was ranged from 2.0×10^1 to 8.4×10^3. 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 (Mckee, 1995; Lin *et al.*, 2005). 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 2.8×10^2 to 8.6×10^6 cfu/g. The fungal count in selected plant materials are similar to those reported previously by Czech *et al*., (2001), Esimone *et al*., (2007), Phianphak *et al*., (2007) and Idu *et al*., (2008). None of the sample showed fungal count under permissible levels (Anon., 1984). 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.

Table 1. Commercially available medicinal plants used in the present study.

Plants	Family	Local names	Parts used	Traditional uses
Acacia nilotica	Leguminosae	Kikar	Flowers & fruits	Used for nerves weakness, diarrhoea, sore throat, malaria, chronic fever, eczema and chest complaints
Berberis lycium	Berberidaceae	Sumbal	Roots	Used as a bitter, tonic, astringent, diaphoretic, febrifuge and to treat broken bones, wounds, jaundice, gonorrhoea, piles, ulcers, opthalmia etc.
Calendula officinalis	Compositae	Gul-e-Ashrafi	Flowers	Used for inflammation, fever, boils, abscesses, wound healing, skin diseases, stomach cramps, ulcers, diarrhoea and vomiting etc.
Carthamus oxycantha	Compositae	Pohli	Seeds	Used for Jaundice. Seed's oil is used for ulcer and against itching.
Cassia absus	Leguminosae	Chaksu	Seeds	Used as a blood tonic, bitter and astringent
Cassia fistula	Leguminosae	Amaltas	Fruits	Used in abdominal pain, constipation, fever, heart disease, and leprosy
Chenopodium murale	Chenopodiaceae	Kurund	Fruits	Used for dyspepsia and Jaundice
Cichorium intybus	Compositae	Kasini	Aerial parts	Used as a tonic, diuretic and to treat fevers and vomiting
Citrullus colocynthis	Cucurbitaceae	Thumba	Fruits	Used as a cathartic, diuretic, emetic, expectorant and to cure jaundice, coughs, inflammations, dropsy, reheumatism and intestinal parasites
Curcuma longa	Zingiberaceae	Haldi	Rhizomes	Used for antibacterial, anti-inflammatory and hepatoprotective activities
Cyperus rotundus	Cyperaceae	Deela ghass	Tubers	Used as a stimulant, astringent, diuretic and stomachic
Dodonaea viscosa	Sapindaceae	Sanatha	Bark & leaves	Used as a febrifuge, astringent and to treat swellings and burns
Ephedra intermedia	Ephedraceae	Asmani booti	Flowers	Used to treat cough, excessive sweating and as a narcotic
Eruca sativa	Cruciferae	Taramira	Seeds	Oil is used for massage and skin infections
Fagonia arabica	Zygophyllaceae	Dama	Shoots	Used for blood purification, fever, cold and cough etc.
Ferula narthex	Umbelliferae	Heeng	Leaves & shoots	Used for asthma, whooping cough, flatulent colic, pneumonia, bronchitis, toothache, abdominal pain and diabetes etc.
Ficus carica	Moraceae	Anjir	Fruits	Used for treating lung and stomach disorders.
Ficus glomerata	Moraceae	Gular	Fruits	Used as an astringent and carminative.
Foeniculum vulgare	Umbelliferae	Saunf	Seeds	Used as a carminative, mouth freshener, and for antimicrobial action.
Fumaria officinalis	Fumariaceae	Shahtra	Aerial parts	Used as laxative, diuretic, alterative, diaphoretic, febrifuge, aperients etc.
Glycyrrhiza glabra	Leguminosae	Multhi	Roots	Used for cough, constipation and abdominal pain.
Linum usitatissimum	Linaceae	Alsi	Seeds	Used for backache and wound's healing.
Mentha longifolia	Labiatae	Jungle podina	Aerial parts	Used for fever, chest disease and chronic diarrhea.
Mentha piperita	Labiatae	Pudina	Seeds	Used to treat headache, sinusitis, chest congestions and stomach disorders.
Nigella sativa	Ranunculaceae	Klonji	Seeds	Used for strengthening immune system and promoting lactation.
Ocimum basilicum	Labiatae	Niazbo	Seeds	Used as a demulcent, expectorant, antiseptic and to treat coughs.
Ocimum sanctum	Labiatae	Tulsi	Seeds	Used as an insect repellent and to treat common cold, stomach disorders and malaria.
Peganum harmala	Zygophyllaceae	Harmal	Seeds	Used as an antispasmodic, hypnotic, emetic, anthelmintic, narcotic and for the cure of rheumatism, coughs, and stomachache etc.
Plantago ovata	Plantaginaceae	Ispaghol	Seeds & husks	Used as a demulcent and for lowering cholesterol, and treating dysentery.
Podophyllum hexandrum	Podophyllaceae	Ban-kakdi	Fruits	Used as a tonic, stimulant, purgative and for the treatment of hepatic problems.
Rheum emodi	Polygonaceae	Rhubarb	Roots	Used as a liver tonic, stomachache, astringent, diuretic, emmenagogue etc.
Ricinus communis	Euphorbiaceae	Arand	Seeds	Used in poultice and to relieve pains. Seeds used in scorpion sting.
Rosa damascena	Rosaceae	Gulab	Flowers	Used to treat abdominal diseases and for tonic and cosmetic properties.
Saccharum arundinaceum	Poaceae	Sarkanda	Stems & roots	Used as a diuretic, diaphoretic, refrigerant and for urinary complaints.
Solanum nigrum	Solanaceae	Mako	Aerial parts	Used as a disinfectant, emollient, febrifuge, narcotic, purgative and sedative etc.
Tagetes erecta	Compositae	Gainda	Flowers	Used as an anthelminitic, aromatic, carminative, diuretic, emmenagogue, laxative, sedative etc. It contains lutein, which is used as a food colorant.
Terminalia chebula	Combretaceae	Hareer	Fruits	Used as an astringent, purgative, stomachache, laxative and antiseptic.
Trachyspermum copticum	Umbelliferae	Ajwain	Seeds	Used for gastric troubles.
Urginea indica	Liliaceae	Jungli-piaz	Bulbs	Used as a cardio-tonic and expectorant.
Urtica dioica	Urticaceae	Bichu Buti	Roots	Used as an anthelmintic and diuretic, and for the regulation of menstrual period and to stop bleeding.
Valeriana jatamansi	Valerianaceae	Mushk-e-bala	Rhizomes	Used as a carminative, aromatic and antispasmodic, and for the treatment of hysteria.
Cataranthus roseus	Apocynaceae	Sadabahar	Flowers	Used as a hypotensive, sedative and for tranquillising and anti-cancerous properties. Used in muscle pain, depression, and wasps stings.
Viola pilosa	Violaceae	Banafsha	Roots	Used as a purgative, tonic, expectorant and diuretic, and used for the cure of malarial fever, bronchitis, asthma etc.
Withania somnifera	Solanaceae	Asgand-e- nagori	Aerial parts	Used as a tonic, alternative, sedative, restorative, diuretic, deobstrust etc.
Xanthium strumarium	Compositae	Gusato	Aerial parts	Used as a sedative, astringent, diuretic and to treat malaria and small-pox.

Table 2. Microbiological profiles of selected plant materials.

Plant	Aerobic bacteria (cfu/g)	Coliforms (cfu/g)	*E. coli* (cfu/g)	*Salmonella* spp. (cfu/g)	Fungi (cfu/g)
Acacia nilotica	2.2×10^5	1.6×10^3	6.3×10^2	–	4.1×10^5
Berberis lycium	1.3×10^2	–	–	–	3.6×10^3
Calendula officinalis	6.2×10^5	–	–	–	9.1×10^4
Carthamus oxycantha	9.4×10^7	2.8×10^3	3.8×10^2	–	5.8×10^4
Cassia absus	5.7×10^3	–	–	9.3×10^2	1.7×10^3
Cassia fistula	4.9×10^5	6.3×10^3	7.9×10^1	–	7.1×10^3
Chenopodium murale	4.0×10^3	–	–	–	9.6×10^4
Cichorium intybus	3.3×10^8	–	–	–	5.5×10^5
Citrullus colocynthis	8.2×10^4	7.2×10^3	5.4×10^2	2.3×10^3	2.8×10^2
Curcuma longa	5.6×10^9	–	–	–	8.3×10^3
Cyperus rotundus	1.9×10^6	1.6×10^4	2.2×10^2	3.4×10^1	2.2×10^4
Dodonaea viscosa	1.6×10^3	–	–	–	5.6×10^4
Ephedra intermedia	8.8×10^8	2.6×10^2	8.2×10^1	–	7.8×10^3
Eruca sativa	3.6×10^6	9.3×10^2	2.1×10^1	–	2.8×10^5
Fagonia arabica	9.5×10^3	–	–	–	5.4×10^4
Ferula narthex	7.6×10^5	3.8×10^3	7.5×10^2	3.9×10^2	1.6×10^3
Ficus carica	5.5×10^6	4.5×10^3	7.3×10^2	–	8.3×10^6
Ficus glomerata	4.0×10^6	8.6×10^3	6.3×10^3	–	2.5×10^5
Foeniculum vulgare	7.2×10^3	–	–	2.7×10^3	3.7×10^4
Fumaria officinalis	7.4×10^6	7.1×10^2	2.8×10^2	–	6.5×10^5
Glycyrrhiza glabra	1.9×10^7	4.6×10^3	2.0×10^1	–	9.4×10^5
Linum usitatissimum	3.2×10^3	–	–	–	3.2×10^6
Mentha longifolia	4.8×10^7	–	–	–	6.0×10^5
Mentha piperita	7.7×10^3	–	–	–	4.9×10^5
Nigella sativa	8.1×10^7	9.7×10^3	1.0×10^2	–	6.6×10^3
Ocimum basilicum	5.8×10^3	–	–	–	7.4×10^3
Ocimum sanctum	8.3×10^6	1.5×10^2	1.1×10^2	1.8×10^3	7.9×10^3
Peganum harmala	7.5×10^3	–	–	–	6.7×10^2
Plantago ovata	1.6×10^5	6.7×10^3	3.6×10^1	–	5.8×10^4
Podophyllum hexandrum	4.9×10^5	4.9×10^3	1.8×10^2	7.4×10^1	6.2×10^5
Rheum emodi	4.2×10^3	–	–	–	2.8×10^3
Ricinus communis	3.9×10^3	–	–	–	2.0×10^5
Rosa damascena	3.6×10^5	2.9×10^3	5.0×10^2	–	2.3×10^4
Saccharum arundinaceum	5.3×10^7	4.6×10^3	5.0×10^2	3.7×10^3	1.6×10^4
Solanum nigrum	1.3×10^5	2.5×10^2	1.4×10^2	1.2×10^2	2.8×10^4
Tagetes erecta	4.8×10^7	8.6×10^3	1.4×10^1	–	8.6×10^6
Terminalia chebula	8.6×10^3	–	–	–	7.7×10^3
Trachyspermum copticum	3.5×10^4	–	–	2.0×10^1	1.0×10^5
Urginea indica	7.4×10^6	3.7×10^3	5.0×10^2	1.9×10^3	5.7×10^4
Urtica dioica	4.8×10^7	–	–	8.4×10^3	1.8×10^5
Valeriana jatamansi	2.2×10^3	–	–	–	3.8×10^5
Cataranthus roseus	7.4×10^3	–	–	–	3.5×10^4
Viola pilosa	3.7×10^7	–	–	6.8×10^2	6.2×10^4
Withania somnifera	4.5×10^5	4.4×10^3	6.7×10^2	–	4.6×10^3
Xanthium strumarium	5.7×10^7	8.5×10^2	2.6×10^1	–	7.3×10^5

Experiment run in triplicate – = not detected

The present studies showed very high microbial loads and the presence of pathogenic organisms in dried medicinal plant materials, commercially available in the city of Peshawar. Traditionally, these plants are used in the form of infusions. There is evidence that the micro-organisms present in plant materials are able to survive the procedures used to prepare infusions (Baxter & Holzapfel, 1982; Katusin-Razem *et al.*, 1988). This potential for survival signified the public health risks especially given that infusions may be prepared using cold water. Other routine household methods for herbal preparations are: hot or cold water extraction, expression of juice after crushing, formulation of powder materials in pastes or mixing with oil or honey etc. Plant materials with high microbial loads are therefore extremely unsafe and hazardous for human consumption. Certain bacteria can tolerate extremes of temperatures or even treatments like extraction with ethanol (Kneifel *et al.*, 2002).

In Pakistan, medicinal plants are not generally cultivated. They grow wild and collected from the hilly areas, barren regions, wastelands, deserts and coastal zones of the country. Manual labour is used to harvest the crops and the plants are then spread directly on lands and sun or shade dried. In market, these materials are kept without any suitable storage conditions and proper packaging. The local collectors, shopkeepers, traders and suppliers do not bother about the cleanness, purity and quality.

Conclusion

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. 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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