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Review

Microbial contamination of medicinal plants

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Abstract

The development of the herbal pharmaceutical industry over the past few years reflects the increased consumer interest in these products, but it also involves new regulatory challenges to appropriately monitor quality, safety and consistency effectiveness of herbal products. Microbial contamination is a danger for the consumer because of the infectious potential of microorganisms that can contaminate medicinal plants. This review attempts to describe certain generalities on the microbial contamination of herbal medicinal products, the admissible limits of this contamination recommended by the World Health Organization and the European Pharmacopoeia, the negative effects of microbial agents contaminating plant- based products and finally a description of certain methods of prevention against microbial contamination.

Keywords:
Medicinal plants, herbal products, Microbial Contamination, side effect

1. Introduction

Medicinal plants have long been used in therapy all over the world and still form an important part of traditional medicine. The oldest method of combating disease is the use of medicinal plants. Also, medicinal plants have also been used for centuries for the aroma and flavor characteristics they impart to foods. These medicinal plants can be either a wild plant or a plant cultivated specifically for medicinal purposes.

Typically, 80% of the world's population depends on herbal medicine [1] and the use of herbal products is seen as an alternative to conventional medicines in several developed countries [2].

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The growing popularity of herbal medicinal products has taken a huge share of the health market [3] because herbal components are considered ”natural” or ”harmless”. Therefore, the trend of herbal medicine research is gaining popularity every day by a large number of pharmaceutical industries for the treatment of various ailments and disorders [4].

Many medicinal plants are grown and harvested under poor sanitary conditions in hot and humid areas which favor potential microbial contamination and degradation of the quality of herbal products. This degradation can cause various infections for consumers and in severe cases can severely damage internal organs with severe dissatisfaction of consumers which can ultimately lead to unwanted financial losses in the pharmaceutical industry. In view of the increased awareness of security in the world, it has become necessary to exercise strict control over the use of herbal products, especially for medicinal purposes [5], [6]. Therefore, the safety of herbal products has become a major concern in public health [5]. Maintaining good microbiological quality in medicinal plants is important to ensure consumer safety.

This review focuses on microbial contamination of medicinal plants and their products with some reports concerning several countries, the admissible limits of this contamination established by the World Health Organization and the European Pharmacopoeia, the negative effects of microbial agents on the quality of plant-based products as well as the health of consumers. Finally, some prevention methods were cited to avoid the harmful effects of this contamination.

2. Generalities

Microbial contamination of medicinal plants can be classified as primary (originating from the microflora of the plant itself) and secondary (due to the handling of plant material). A wide variety of bacteria, fungi and viruses can be found in or on plant material [7]. The most likely sources of contamination are microbes in soil and treatment facilities, and some preparations may contain foreign material and dirt from rodents, insects, inorganic source, human faeces and animal manure [6]. Atmospheric and terrestrial vectors can play a role in microbial contamination [7]. The methods of harvesting, drying, storage, transport and processing also affect the quality of the plants [8]. The microbial load is relatively dependent on temperature, humidity, handling and storage practices of raw and processed medicinal plant materials [9] [7]. Uncontrolled humidity during storage promotes the development of fungi that contaminate plants after harvest [10]. Extraction at room temperature allows microbial multiplication and extraction with cold water favors the accommodation of a considerable quantity of microbes, on the other hand extraction with boiling water considerably reduces the quantity of microbes and inactivates pathogens Extraction with ethanol or methanol can provide also a good hygienic condition [7]. Very large amounts of bacterial spores and molds can be carried to medicinal plants by environmental dust and other contaminations [11]. Bacterial endospores and fungal spores are the dominant microbial contaminants of medicinal plants [7]. The spores are mainly found on the dry plant substance and are very resistant to chemicals and different environmental conditions and are the most common cause of food poisoning [9] [12] [13]. The presence of Enterobacteriaceae and E. Coli reflects fecal contamination [14]. The presence of coliforms is an indicator of undesirable hygienic conditions [7]. In most developing countries where good agricultural practices and good harvesting practices are not well implemented, there is a greater chance of contamination by toxigenic fungi that originate from the soil [15] [16]. Also, the conditions of transport and storage have an influence on the fungal contamination of herbal medicines [17]. Fungal contamination requires great attention because, it can produce mycotoxins [7] [18]. which are toxic secondary metabolites produced by molds such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* spp. [19] [20] [16]. Mycotoxins cause various toxic effects, such as carcinogenic, neurotoxic, teratogenic and immunotoxic effects and can be produced or secreted during plant growth or during storage of herbal products [21] [12]. The most important mycotoxins are aflatoxins since there are almost 20 different types of aflatoxins of which the four main ones are aflatoxins B1, B2, G1 and G2 . aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) are produced by *Aspergillus* flavus, while the four isoforms (B1, B2, G1, G2) are produced by *Aspergillus* parasiticus [12] [22]. AFB1 is considered the most toxic mycotoxin. Plant substances from plants grown in a (sub)tropical climate can exhibit significantly higher aflatoxin levels than those grown in cooler and drier climates [12]. Ochratoxin (OT) is another family of mycotoxins produced by many species of *Aspergillus* and *Penicillium* [23][24]. This family consists of three members, A, B and C, OTA is the most abundant and toxic of the three, while ochratoxin B (OTB) and ochratoxin C (OTC) are less important and less common [25].

3. Admissible limits of microbial contamination

With regard to microbial contamination, the limits recommended by the World Health Organization for the contamination of raw plant material intended for further processing are mold and yeast (10^5 cfu/g) and E. coli (10^4 cfu/g); for plant materials that have been pretreated or are used as topical dosage forms, the recommended limits are aerobic bacteria (10^7 cfu/g), mold and yeast (10^4 cfu/g) E. coli (10^2 cfu/g), enterobacteria and other Gram negative bacteria (10^4 cfu/g) as well as salmonella must be absent; for other plant materials for internal use, the recommended limits are aerobic bacteria (10^5 cfu/g), mold and yeast (10^3 cfu/g), E. coli (10 cfu/g), enterobacteria and other Gram negative bacteria (10^3 cfu/g) and salmonella must always be absent [26]. According to the European Pharmacopoeia and for herbal medicines to which boiling water is added before use, the recommended limits are aerobic bacteria (10^7 cfu/g), mold and yeast (10^5 cfu/g) and E. Coli (10^3 cfu/g), and for herbal medicines to which boiling water is not added before use, the recommended limits are aerobic bacteria (10^5 cfu/g), mold and yeast (10^4 cfu/g) and enterobacteria and other Gram-negative bacteria (10^3 cfu/g) with the absence of salmonella and *E. coli* [27].

4. Microbial contamination reports

In Iran, the study of the contamination of 35 samples of plant water and 35 samples of dried medicinal plants of 7 species (lavender, lemon balm, valerian, savory, borage, mint and thyme) (total samples = 70) showed that all samples of plant water were contaminated with mesophilic bacteria and yeasts based on Iranian national standard. However, or but, none of the dried medicinal plants were contaminated according to the Iranian national standard [28]. In Bangladesh, microbiological analysis of 125 samples of oral herbal medicines showed that out of 85 oral liquid samples, two were found to be highly contaminated with a total aerobic bacterial load of 1.24×10^5 cfu/ml, 10 samples were contaminated by fungi (1.2×10^4-6.3×10^4 cfu/ml), one sample was contaminated with coliforms but none of the samples were contaminated with Salmonella spp. and Shigella spp. Also, among 40 semi-solid samples, one was found to be contaminated with bacteria (1.93×10^5 cfu/g) and 5 samples consisted of a fungal load between 1.5×10^4-2.2×10^4 cfu/g [29]. In a study carried out in Pakistan, 45 commercial medicinal plants were evaluated for their microbial contamination and the results showed that the number of aerobic bacteria ranged from 1.3×10^2 to 5.6×10^9 cfu/g, the number of coliforms varied from 1.5×10^2 to 1.6×10^4 cfu/g and the fungal counts were above the internationally permitted level. In addition, 23 samples showed the presence of E. Coli and 13 samples were contaminated with Salmonella spp. [30]. In Iraq, 16 samples representing different types of spices and medicinal plants were collected and analyzed to shed light on their microbial status. Ten different fungi genera and 16 species have been isolated and identified as *Alternaria* alternata, *Aspergillus* spp., Gliocladium sp., Hyalodendron diddeus, Memmoniella sp., *Penicillium* spp., Rhizopus spp., Syncephalastrum sp., Cladosporium lignicolum and Ulocladium botrytis. *Aspergillus* spp. and *Penicillium* spp. were more frequently detected, while Stachybotrys sp., Syncephalastrum racemocum, Uocladium botrytis, *Alternaria* alternata, Cladosporium lignicolum and Gliocladium catenulatum were less frequently detected. A. flavus, A. Niger and A. ochraceous show positive results for the production of mycotoxins. For the estimation of the natural occurrence of aflatoxin (AT) and ochratoxin (OT) in selected dried samples; the high level of aflatoxin and ochratoxin appears in Red tea (150.5, 387.3 ppb) while their low level has been observed in Garlic (1.4, 0 ppb) respectively [31].

In Kenya, examination of the microbiological quality of 24 herbal products marketed to retailers in Nairobi showed that total aerobic mesophilic flora (FTAM) ranged from 1.5×10^1 to7.1×10^8 cfu/g, the number of total and faecal coliforms ranged from ¡10 to 3×10^6 cfu/g, the load of E. Coli ranged from ¡10 to 5×10^1 cfu/g, the number of S. aureus ranged from approximately ¡10 to 2.5×10^3 cfu/g, and the number of yeasts and molds ranged from approximately ¡10 to 9×10^4 cfu/g., the percentage of samples contained microorganisms above the permissible limits according to WHO were: 33% (APC), 50% (coliforms) and 33% (yeasts and molds) [32]. In another study, microbiological analysis (total viable aerobic count, enumeration of yeasts and molds, detection of Enterobacteriaceae, other Gram- negative bacteria and Staphylococcus aureus) of 72 samples of medicinal plants produced in different regions of Brazil showed that the majority of samples (79%) do not agree with the World Health Organization parameters for herbal medicines to be used for herbal teas or topical and internal uses due to the high number of aerobic organisms and yeasts and molds [33]. In Portugal, the evaluation of the microbial contamination of 62 samples of seven medicinal plants (chamomile, leaves of orange tree, flowers of linden, corn silk, marine alga, pennyroyal mint and garden sage) was studied; the results showed that 96.8% of the samples were contaminated with Bacillus cereus and 19.2% of them with levels above 103 spores/g. Clostridium perfringens spores were detected in 83.9% of the samples but only 19.2% had levels above 10^3 spores/g. The average level of fungal load was $10^{5.5}$ cfu/g. *Fusarium* spp., *Penicillium* spp., *Aspergillus* flavus and *Aspergillus* niger was predominant in all samples except the garden sage samples. Many yeasts have been found in chamomile, flowers of linden, corn silk, pennyroyal mint and garden sage. The predominant species were Cryptococcus laurentii (28.1%) and Rhodotorula mucilaginosa (22.8%) [11].

5. Negative effects of microbiological contamination

Fungal contamination decreases the medicinal potency of medicinal plants by adversely affecting the chemical composition of raw materials [34]. Exposure of medicinal plants to water results in the loss of bioactive molecules under the influence of colonizing fungi [35]. Even at low temperatures, there may be loss of active compounds through spontaneous biodegradation by microbial contaminants during storage of herbal medicines [36].

Staphylococcus aureus is most likely to arise from contact with herbal handlers during harvest, processing and storage, and its absence reflects good hygienic practice. Contamination with Staphylococcus aureus could supply an amount of enterotoxin, which also causes food poisoning [6]. Escherichia coli is a foodborne pathogen and considered an indicator of faecal contamination, and is frequently found in soil and water contaminated with human sewage or animal manure [37] [38]. Also, the ability of Salmonella to survive drying treatments and subsequent storage in products with low water activity increases the risk of infection [13]. In addition, outbreaks of salmonellosis associated with the consumption of contaminated spices or herbs have been reported [39].

Low levels of contamination with Bacillus cereus and Clostridium perfringens in medicinal plants are common due to the natural habitat in the soil environment, and both of these bacterial species are potential pathogens that can cause severe food poisoning when ingested in large numbers [40].

Aflatoxin-producing toxigenic molds are very dangerous since these mycotoxins are carcinogenic, teratogenic, genotoxic and mutagenic [5]. Aflatoxin typically produced by A. flavus is responsible for large economic losses [41].

6. Decontamination and sterilization of herbal products

Chemical or microbial contamination of a raw material, intermediate or finished plant-based product may occur during production, sampling, packaging or repackaging, storage or transport, so quality control in all these steps is essential [5]. To reduce microbial contamination, a high level of sanitation and hygiene during manufacturing is necessary [42]. Good manufacturing practices are certainly essential steps for the quality control of medicinal plants[15]. Manufacturers must ensure the lowest possible level of microorganisms in herbal products and in packaging components in order to maintain appropriate quality, safety and efficacy of natural products [43] [44].

Drying can lower water activity and inhibit the growth of fungal species and also ensure the quality of medicinal plants [45]. Also, adequate extraction methods help reduce microbial contamination and achieve microbial limits specified by pharmacopoeias [43].

Microbial decontamination and sterilization are methods of inactivating microorganisms, especially pathogens. Microbial decontamination is seen as the removal or reduction in the level of microorganisms while sterilization is defined as the complete destruction, inactivation and removal of all microorganisms [46].

There are many different decontamination and sterilization methods, each with specific characteristics and applicability.

Sterilization is achieved either by exposure to a physical or chemical agent for a predetermined period or by physical removal of the organisms. The hostile agents used are high temperatures, ionizing radiation or chemicals in the form of gas, plasma gas or liquid [47] [48]. Physical removal involves passing solutions or gases through a filter capable of retaining microorganisms [47] [49]. The chemicals cause the reduction of the aroma of herbal products by attacking the heat-sensitive components of the plant material but these techniques have an effective decontamination approach [50]. The main reason for using decontamination methods is to increase the shelf life of the herbal medicine.

Fumigation is the technique in which plant material is treated with a gaseous compound that kills microorganisms (bacterial, fungal or mold) with doses of direct action [51] [52]. Also, freeze-drying helps to reduce the weight and volume of the plant with positive consequences for transport and storage [9]. Lyophilization has unforeseen and potentially significant effects on the constituent profiles and medicinal action of plants [53].

High pressure processing (HPP) is a method where the material is subjected to high pressures applied with a pressure transmitting medium (water or other liquids)[12]. It is a non-thermal technology that destroys unwanted microorganisms and inactivates enzymes without affecting bioactive constituents. It is also less likely to lose volatile compounds [54]. The HPP technique based on a pressure ranging from 100 to 800 MPa without any involvement of heat can cause microbial decontamination (bacterial, yeast, mold and virus) [55]. Pressure reaches inactivation of vegetative microorganisms and factors leading to cell death include unfolding or denaturing of proteins, phase transition of cell membranes, disintegration of ribosomes, and changes in intracellular pH resulting in inactivation or damage to the membrane [56].

The different methods of irradiation include gamma rays, electron beam and X- rays [13] [57], these ionizing radiations attack the DNA strands of microorganisms making them fatally damaged and due to which they are unable to replicate and become nonviable [57]. Flowers, leaves and liquid extracts are contaminated more quickly than fruits, seeds and dry plant matter and the order of irradiation resistivity follows the order of leaves¿ fruits¿ seeds¿ liquid extracts¿ dry extracts [58]. The radiation sensitivity of microorganisms in plant products depends mainly on the nature of the microorganism, the initial microbial load, the chemical composition, the physical state of the product, the irradiation parameters, the temperature and the presence or the absence of oxygen [59] [60] [61].

Gamma irradiation is used for product sterilization and microbial load reduction in food segments, packaging, medical devices, pharmaceuticals, cosmetics and veterinary products [62] [63]. Also, this method is commonly used for the sterilization of active components and drug delivery systems [64]. Irradiation with gamma rays causes breaks in the chemical bonds of DNA with the release of free radicals which attack the nucleic acid of microorganisms [65]. This method guarantees the quality and safety of the products and it leaves no residue [65]. Therefore, gamma irradiation is considered to be an effective treatment for the microbial decontamination of medicinal plants .

7. Conclusion

According to the data in this review, microbial contamination of medicinal plants is inevitable and especially under certain cultivation and collection conditions. In order to ensure consumer safety, the World Health Organization and the European Pharmacopoeia have established admissible limits for the microbial contamination of medicinal plants and their products. According to the reports of our review, several pathogens have been reported to contaminate medicinal plants and their products, these microbial agents having the capacity to cause foodborne illness in consumers. Several decontamination methods have been shown to be effective in decontaminating medicinal plants and their products in order to ensure the safety of consumers.

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