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Novel decontamination approaches for stability and shelf-life improvement of herbal drugs: A concise review

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ABSTRACT

The market for herbal drugs has risen enormously in the current era, and it has led to the formulation of numerous medicinal herbs. However, many of the drug components may be affected by microbial metabolites, which have led to possible concerns regarding the stability and shelf-life of such formulations. Herbal products are frequently affected by degradation, particularly during storage, which can lead to loss of active constituents, synthesis of inactive metabolites, and production of toxic metabolites. These aspects need to be addressed in order to regulate the stability and efficacy of herbal drug formulations. In particular, pathogenic microorganisms, especially those able to form biofilms, can derive from different environments at various stages of herbal drug preparation. Thus, the stability and shelf-life could be improved by preventing microbial contamination of medicinal herb products during preparation, packaging, storage, and delivery. This review deals with problems induced by microbial pathogens and their biofilms and proposes novel decontamination approaches to reduce microbial contaminations to increase the stability and shelf-life of medicinal plants and their formulations.

1. Introduction

The usage of therapeutic herbs is constantly growing worldwide due to their reduced risk of side effects (Welz et al., 2018). A surge in the search for herbal remedies is due to the spread of diseases that go untreated and the advancement of scientific understanding of herbal medicines as effective therapy alternatives (Ekor, 2014). As a result, there is a great deal of worry concerning the efficacy and safety of herbal treatments, and one of the key factors in determining the grade of research on the effectiveness of herbal drugs is the quality of the plant materials (Balekundri and Mannur, 2020). Herbal medicines must be of a certain quality to be safe, effective and reproducible (Pelkonen et al., 2014; Kumari and Kotecha, 2016; Balekundri and Mannur, 2020). Furthermore, due to the ever-rising use of herbal medicines and the industry's global diversification, safety has become a worry for both the general population and the health authorities in many nations (Mensah et al., 2019; de Sousa Lima et al., 2020). The effectiveness of herbal products, as well as consumer health, may be adversely affected by microbial contaminants (*Salmonella* spp., *S. aureus*, *Shigella* spp., *P. aeruginosa, E. coli, A. parasiticus, A. flavus, A. niger and A. ochraceus*) and microbial residues such as aflatoxins virulence proteins and other toxic metabolites (WHO, 2007; Mensah et al., 2019; de Sousa Lima et al., 2020). Likewise, these pathogenic microbes contaminate the medicinal herbs and colonize the surfaces or inside the plants for an extended period of time. Therefore, microbiological contamination in non-sterile pharmaceutical products has the ability to lessen or perhaps neutralize the therapeutic efficacy and have an adverse effect on the patients who receive the drugs (Ratajczak et al., 2015; Ahmad et al., 2023). As complex mixtures are generated from biological sources, herbal medications take much work to achieve a constant and appropriate quality.

Microbial contamination in herbal remedies may be caused by medicinal plants' microbiological (bacterial and fungal) load. Temperature, soil pH, heavy metals, air quality, and precipitation are all biotic and abiotic factors that can influence their existence. The microbiological (bacterial and fungal) load of medicinal plants may be the source of microbial contamination in herbal medicines (de Sousa Lima et al., 2020). In particular, medicinal plants are highly susceptible to contaminations by pathogenic microorganisms (*S. aureus*, *P. aeruginosa,*

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Shigella spp., *Salmonella* spp., *E. coli*, *A. parasiticus, A. flavus, A. niger and A. ochraceus*) from soil, water and air (Castronovo et al., 2021). These microbes can reduce the shelf-life and quality of the drugs, and they may be potentially harmful to humans (Yesuf et al., 2016; Taghinasab and Jabaji, 2020). Pathogenic microbes constitute mono and multispecies biofilms on herbal drugs and reduce their potency. Microbial contamination of medicinal herbs can also be affected by climatic conditions during the handling procedures, pre- and post-harvesting stages, and storage conditions of fresh and processed medicinal plant resources such as humidity, temperature, pH and amount of rainfall (Abba et al., 2009; de Freitas Araújo and Bauab, 2012; de Sousa Lima et al., 2020). To improve the safety, efficacy, purity, and shelf-life of the drugs, observation of essential cleanliness and sanitization procedures are required to ensure microbial contamination below the permissible levels, such as standardization of pH, moisture content, and durability (Mugoyela and Mwambete, 2010; Khemuka et al., 2015). However, the microbiological quality of the herbal drugs can be influenced by modifications and processing causes that occur when medicinal herbs are harvested and further preparations for drugs such as extraction, formulations, etc (Ratajczak et al., 2015; Patel et al., 2019). Previous reports have confirmed the existence of promising microbial contaminants (*S. aureus*, *Shigella* spp., *E. coli*, *Salmonella* spp., *P. aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Klebsiella oxytoca, A. parasiticus, A. flavus, A. niger* and *A. ochraceus*) in the formulation of some herbal drugs, prepared from various medicinal herbs such as *Brassica hirta, Hydrastiscan adensis, Zingiber officinale, Rosmarinus officinalis, Allium sativum, turmeric, Curcuma longa, Allium sepa,* and *Brassica juncea* (Ratajczak et al., 2015; Ratajczak et al., 2020; de Sousa Lima et al., 2020). Thus, considering microbial contamination as a significant threat in herbal drugs, manufacturers should ensure the minimum possible number of microbes in the fresh plant materials, completed dosage forms, and packaging modules to sustain adequate safety, quality, better shelf-life, and efficacy of the herbal medicines.

This review aims to summarize advances in understanding microbial contaminations of medicinally important herbs and herbal drugs by deliberating the impact of regularly used pharmaceutical preparation procedures to reduce the microbial contaminants in herbal drugs. Moreover, quality and shelf-life standards are conferred, deliberating the guidelines of microbiological quality control and quality assurance processes such as GMP (good manufacturing practices) for herbal drugs.

2. Bibliometric analysis

Bibliometric analysis of the subject based on Google Scholar using five keywords ("medicinal plant" and "microorganisms," "microorganisms" and "shelf-life," "microorganisms" and "herbal products" "microorganisms" and "decontamination techniques," "microbial biofilms" and "herbal products") between 1900 –2022 revealed an expansion in methodical research on the proposed theme, only after 2010. In contrast, very little research on these themes could produce any news in the scientific field before the year 2000. Of 439 publications (research and reviews), a maximum of 337 publications dealing with stability and shelf-life, followed by 80–101 publications wrapping the subjects like microbial contamination or microbial load and strategies to prevent microbes, intensely showcasing the chronological shifts in research interest globally. The increased availability of reasonably priced technology can be attributed to the surge in publications following 2010. A schematic representation of bibliometric analysis is provided in figure S1.

3. Possible human pathogens associated with medicinal plants

The traditional and modified modern plant breeding methods for growing and harvesting of medicinal plants cannot completely prevent microbial contamination of the plant material, which can be derived from the surrounding environment and the hygiene applied throughout various treatments like field treatments and industrial preparation (Gil et al., 2015; Amit et al., 2017). Biological contaminations may encompasses of communities of bacteria (*S. aureus*, *Salmonella* spp., *Shigella* spp., *Klebsiella oxytoca, Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterobacter cloacae,*), fungi (yeasts: *Candida* and *Cryptococcus*, and molds: *Aspergillus* spp. and *Penicillium* spp.), oomycetes (*Plasmopara viticola, Phytophthora cinnamomi, Phytophthora parasitica, Phytophthora infestans, Phytophthora ramorum, Phytophthora sojae, Phytophthora capsici,* and *Hyaloperonospora arabidopsidis*), protozoa (Naked amebae, testate amebae, flagellates, ciliates, microsporidia, and sporozoans), viruses (Tobacco mosaic virus, cucumber mosaic virus, tomato yellow leaf curl virus, potato virus Y, African cassava mosaic virus, tomato spotted wilt virus, broccoli mosaic virus, and cauliflower mosaic virus), or insects (Leaf miners, Whiteflies, Earwigs, Cutworms, Aphids, Thrips, Spider Mites, Earwigs, and Mealybugs eggs and larvae) (Kneifel et al., 2002; Degaga et al., 2022) (Fig. 1). Moreover, several toxic compounds released by bacteria (secondary metabolites and virulence factors, such as endotoxins, adherence factors, capsules, invasion factors, exotoxins and siderophores) and fungi (mycotoxins such as aflatoxin and other low molecular weight metabolites, such as ochratoxin A, patulin, fumonisins, zearalenone and nivalenol/deoxynivalenol) considered as major chemical contaminants in herbal drugs (Loi et al., 2020; Awuchi et al., 2021). The possible mycotoxins in medicinally important plants and their deleterious impact on human health are illustrated in Table 1. In particular, the occurrence of spore-forming bacteria, especially *Bacillus* spp. and *Clostridium* spp., are responsible for the formation of endospores in aerobic and anaerobic environments, which are highly resistant to industrial treatments of herbal drug preparation such as heat (Awuchi et al., 2021). Specifically, these two spore-forming bacterial species (*Bacillus cereus* and *Clostridium perfringens*) have been well recognized for their severe pathogenicity for food infection and poising (Mathot et al., 2021; Yehia et al., 2022), and they were recovered from chamomile and other medicinal plants (Yehia et al., 2022). Similarly, members of the Enterobacteriaceae family are commonly found in nature, and some of them (*E. coli*) are indications of the extent of faecal contamination in the environment (Jang et al., 2017). Moreover, the group of coliforms (*E. coli, Klebsiella pneumoniae, Klebsiella aerogenes, Enterobacter cloacae, Klebsiella variicola, Citrobacter freundii, Klebsiella oxytoca, Citrobacter koseri, Klebsiella granulomatis, Pseudescherichia vulneris, Escherichia fergusonii, Escherichia albertii, Enterobacter cowanii, Citrobacter europaeus, Citrobacter portucalensis, Klebsiella huaxiensis, Citrobacter pasteurii, Klebsiella terrigena, Hafnia alvei* and *Klebsiella singaporensis*) and total aerobic mesophilic bacteria are used as a marker of poor hygiene practices (Mathot et al., 2021; Yehia et al., 2022), and therefore this statement must be tied to the quantity of viable count measured in herbal drugs (Yehia et al., 2022). In contrast, *Staphylococcus aureus* is regarded as a common bacterial contamination in medicinal plant materials (Mathot et al., 2021), even though, based on the characteristics of the microbial isolate, it is capable of synthesizing enterotoxins (Argudín et al., 2010; Jang et al., 2017). Herbal drugs are likely to accommodate a variety of other potentially harmful microorganisms, such as *Shigella* spp. and *Salmonella typhi*, in addition to *S. aureus* and *E. coli* (Abba et al., 2009; de Sousa Lima et al., 2020). Additionally, the occurrence of pathogenic bacteria including *B. cereus, Pseudomonas aeruginosa, Shigella spp., Aeromonas hydrophila, Enterobacter agglomerans, Vibrio fluvialis, E. cloacae, Pasteurella multocida, Acinetobacter iwoffii, S. epidermidis, B. subtilis* and *Klebsiella spp.* and fungus (*Rhizopus stolonifera*) were also detected in medicinal plant models (Idu et al., 2011; de Sousa Lima et al., 2020).

Molds are considered distinctive natural contaminants of medicinal plants, and several fungi can synthesize toxic compounds (mycotoxins) which are very harmful to human health (Ałtyn and Twarużek, 2020). Aflatoxin is one of the most poisonous mycotoxins and is usually produced by *A. parasiticus* and *A. flavus*, and other minor fungi. It significantly impairs the quality and effectiveness of herbal drugs (Omotayo et al., 2019; Ráduly et al., 2020). The widely accepted aflatoxin

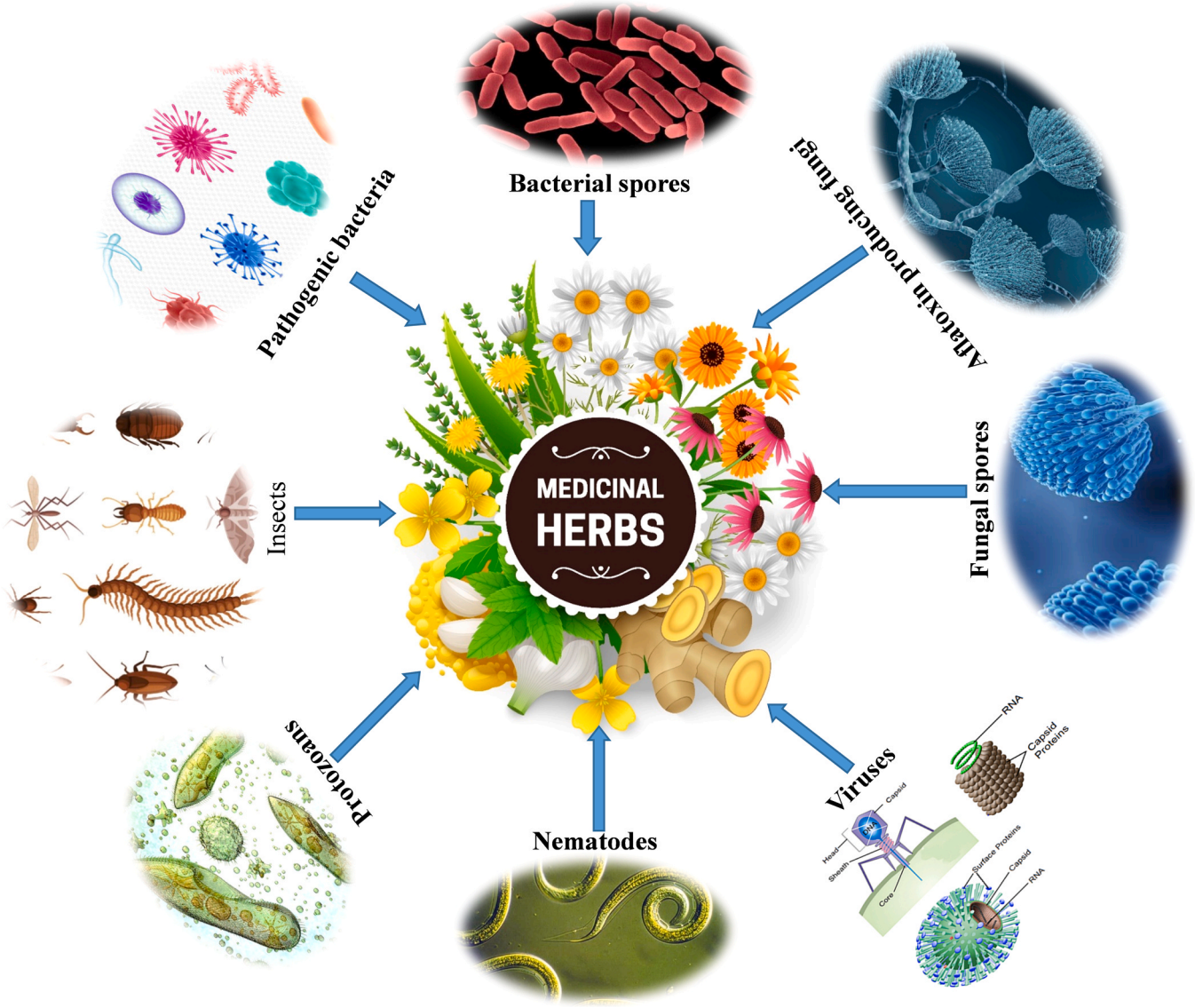

Fig. 1. Possible prokaryotic and eukaryotic contaminations from the environment to medicinal plants.

producer is *A. flavus*, which frequently contaminates herbal tea and other indispensable medicinal plants (Kumar et al., 2017). In an investigation of 91 medicinal plants, fungal contamination was most prevalent (50%) on aerial parts (Ráduly et al., 2020). The samples of medicinal plants (*Amomi fructus, Jujubae fructus, Lycii fructus, Codonopsis pilosula, Notoginseng radix et rhizome, Scutellaria lateriflora Morinda officinalis, Polygoni multiflori, Poria cocus, Coicis semen, Tremella fuciformis, Ganoderma lucidum* and *Lentinus edodes*) were examined for fungal contamination by pioneer worker, and findings revealed that the predominantly 89.9% mycoflora correlated to genera *Penicillium* and *Aspergillus* which are tremendously essential from the perspective of mycotoxicology (Chen et al., 2020). Moreover, fungal contamination of medicinal plants (*Codcnopsitis pilosulas, Rhizoma glycyrrhizae, Radix notoginseng, Armeniacae amarae, Magnoliae officinalis, Fritillariae cirrhosae and Lonicerae japonicae*) are represented mainly by *Penicillium* spp. and *Aspergillus* spp., and other are also present in negligible amounts, such as *Candida* spp., *Mucor* spp. and *Trichosporium* spp. (Zheng et al., 2017; Yu et al., 2020). The interference of fungal toxins adversely deteriorates the chemical constituents of the raw and fresh materials and impairs the therapeutic efficacy of herbal drugs (Zheng et al., 2017; Chen et al., 2020).

The presence of microbial communities in medicinal plants is a serious concern, and standard monographs set a maximum fungal contamination limit for the herbal drugs containing raw materials of natural origin to defined threshold limits (Table 2) in order to ensure the quality, shelf-life, efficacy and safety of these products (Chen et al., 2020). These restrictions are established by considering the natural microflora, current manufacturing techniques and the necessity (Oluyemisi et al., 2012; Patel et al., 2019). A maximum fungal load may be considered because of the natural origin of those products, as these loads reveal the main factor in mycotoxigenesis and herbal medicine spoilage (Patel et al., 2019).

4. Conventional and modern methods for the detection of mycotoxins producing fungi

There are several conventional and modern approaches to identify the level of mycotoxins and their causal organisms in herbal products. The best-known conventional methods are thin-layer chromatography (TLC), total liquid chromatography, and modified culture media (Aspergillus Differentiation Medium Base), which are utilized for more accurate studies and may also be used for screening with visual or densitometric detection of mycotoxin level and responsible fungi (Lin et al., 1998). Nowadays, herbalists use modern and sophisticated

Table 1
Various mycotoxins and their causal organisms associated with medicinal plants in natural ecosystems.

Toxic effects and diseases	Responsible mycotoxins and causal pathogens	Associated plants for contamination	References
Immunotoxic, carcinogenic, hepatotoxic, (decreasing immune systems, affecting the structure of DNA, hepatitis, bleeding, kidney lesions)	Aflatoxin (AF); *Aspergillus*	Liquorice root, Green tea, Ginkgo biloba, Milk thistle, Ginger, Ginseng, Ginseng root, Mint and Chamomile flower	Wang et al., 2013; Ahmad et al., 2014; Martínez-Domínguez et al., 2016; Martinez-Dominguez et al., 2015; Tournas et al., 2012; Tosun and Arslan, 2013; Wen et al., 2014; Trucksess and Scott, 2008; D'Ovidio et al., 2006; Su et al., 2018; Luo et al., 2018
nephrotoxic, carcinogenic, hepatotoxic, immunotoxic, (kidney and liver damage, loss of appetite, nausea, vomiting,suppression of immune system, carcinogenic)	Ochratoxins (OTA, OTB, OTC); *Aspergillus* and *Penicillium*	Green coffee, Grape, Brewer's yeast, Ginger, Ginseng, Mint, Chamomile flower and Liquorice root	Tournas et al., 2012; Solfrizzo et al., 2015; Gottschalk et al., 2016; Wen et al., 2014; Trucksess and Scott, 2008; Santos et al., 2009; Wang et al., 2013; Ahmad et al.,
neurotoxic, immunotoxic (skin necrosis, hemorrhage, anemia, granulocytopenia, oral epithelial lesions, GIS lesions, hematopoietic, alimentary toxic aleukia (ATA), hypotension, coagulopathy)	Trichothecenes (type A and B); *Fusarium, Myrothecium, Stachybotrys, Trichoderma*	Ginkgo biloba, Milk thistle, Mint and Chamomile flower	Martinez-Dominguez et al., 2015; Veprikova et al., 2015; Santos et al., 2009
oestrogenic, immunotoxic, teratogenic (hormonal imbalance, estrogenic effect and reproductive problems)	Zearalenones (ZEN, α-ZOL, β-ZOL, ZAN); *Fusarium*	Different plants, Ginger, Milk thistle, Mint and Chamomile flower	Veprikova et al., 2015; Koul and Sumbali, 2008; Arroyo-Manzanares et al., 2013; Santos et al., 2009; Luo et al., 2018
immunotoxic, carcinogenic, hepatotoxic, nephrotoxic, neurotoxic (encephalomalacia, pulmonary edema, carcinogenic, neurotoxicity, liver damage, heart failure, esophagealcancer in humans)	Fumonisins (FB1, FB2, FB3); *Fusarium*	Green coffee, Milk thistle, Mint, Chamomile flower and Liquorice	Vaclavik et al., 2013; Santos et al., 2009; Luo et al., 2018

Table 2
Recommended microbial load (CFU/gm) for herbal drugs/products in accordance with different established Pharmacopoeia.

Microbial limit type	Indian Pharmacopoeia[a]	WHO[b]	United States Pharmacopoeia[c]	European Pharmacopoeia[d]
Aerobic bacteria	10^5 / 10^4 / 10^2	* / 10^7 / 10^5	10^5 / 10^4 / 10^2	10^7 / 10^5
Yeast and mold	10^3 / 10^2 / 10	10^5 / 10^4 / 10^3	10^3 / 10^2 / 10	10^5 / 10^4
Enterobacteria and other Gram-negative bacteria	10^3 / * / *	* / 10^4 / 10^3	10^3 / * / *	* / 10^3
E. coli	Absent (20 g in 90 ml)	10^4 / 10^2 / 10	Absent (20 g in 90 ml)	10^3 / absent (20 g in 90 ml)
Salmonella spp.	Absent (20 g in 90 ml)	n.d. / absent / (20 g in 90 ml)	Absent (20 g in 90 ml)	n.d. / absent (20 g in 90 ml)
Shigella spp.	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)
P. aeruginosa	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)
Gram-positive bacteria				
Bacillus spp.	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)
S. aureus	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)	Absent (20 g in 90 ml)

a Indian Pharmacopoeia (https://www.ipc.gov.in): the first value denotes contamination of crude plant material intended for further processing; the second value represents for plant materials that have been pretreated with boiling water as used for herbal teas and infusions or that are used as topical dosage formst The third value represents infusions/decoctions.
bFor other plant materials for internal use, (WHO; https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/quality-control/quality-control-methods-for-medicinal-plant-materials.pdf?sfvrsn=b451e7c6_0): the first value indicates contamination of crude plant material intended for further processing; the second value represents for plant materials that have been pretreated with boiling water as used for herbal teas and infusions or that are used as topical dosage forms; the third value represents infusions/decoctions
c For other plant materials for internal use, United States Pharmacopoeia (https://www.usp.org): the first value represents dried or powdered botanicals and botanicals to be treated with boiling water before use; the second value represents tinctures, powdered botanicals extracts, fluid extracts and nutritional supplements with botanicals; the third value represents infusions/decoctions
[d] European Pharmacopoeia (https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-10th-edition-): herbal medicinal products consisting solely of one or more herbal drugs (whole, reduced or powdered): the first value represents herbal medicinal products to which boiling water is added before use; The second value represents herbal medicinal products to which boiling water is not added before use.
n.d. (not determined), when microbial limits are not specified.

techniques to quickly detect mycotoxins in herbal drugs/products. Various methods, including HPLC, LC-MS, Fluorometer, FTIR, RIA, ELISA, Immunodipstic, QCMs, SPR, OLWS, and electrochemical, are applied for the quick detection of mycotoxins in the herbal materials. The previously published review has collectively elaborated all the modern approaches discussed (Wacoo et al., 2014).

5. Biofilms in the industrial production of herbal drugs

Biofilms are organized microbial assemblages and clusters covered by self-producing extra-polymeric substances (EPSs) in a network or meshwork-like matrix (Ansari and Ahmad, 2018). In herbal formulations, biofilms frequently occur among bacterial and fungal communities depending upon the nature of the herbal manufacturing environment. The robust colonizing bacterial species are as follows: *Pseudomonas* spp., *Geobacillus stearothermophillus*, *S. aureus*, *E. coli*, *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. (Muhammad et al., 2020). For instance, biofilms in herbal formulations may comprise the *Pseudomonas* spp., *Geobacillus stearothermophillus*, *S. aureus*, *E. coli*, *Salmonella* spp. and *Klebsiella* spp., causing major health and economic issues (Galie et al., 2018; Muhammad et al., 2020). The existence of multiple bacterial species in biofilm has vital ecological benefits because it can promote adherence to a surface (Galie et al., 2018). Moreover, biofilms composed of multiple microbial species exhibit greater resistance to antiseptics, disinfectants, and sterilizers, including quaternary ammonium compounds and other biocides, including alcohol, hydrogen

peroxide, chlorine and chlorine compounds, glutaraldehyde, formaldehyde, iodophores, peracetic acid and ortho-phthalaldehyde (Ansari and Ahmad, 2018; Yin et al., 2019). The extracellular matrix of biofilm (network and meshwork-like structure) mainly consists of polysaccharides, exogenous DNA and proteins (Ansari and Ahmad, 2018), and it plays an imperative role in adherence of bacterial cells to biological surfaces (medicinal plants) and solid surfaces (e.g. industrial equipment, storage, dispensing and transport surfaces, soil particles) of herbal drug production and delivery (Bogino et al., 2013; Gudynaite et al., 2022). Biofilms can develop rapidly in the herbal drug industry because herbal products are rich in essential nutrients (Yin et al., 2019). Moreover, the extracellular matrix of biofilm plays a protective structure for bacterial cells (Ansari and Ahmad, 2018), and it can increase the persistence of microbial contaminants in herbal drug production (Muhammad et al., 2020). For example, biofilms provide strong protection against chemicals (toxic chemicals, disinfectants, and antimicrobials applied in the industry), physical resistance against desiccation, and mechanical resistance to liquid streams in herbal drug production (Galie et al., 2018; Yin et al., 2019). Moreover, some of the human pathogens (*E. coli, Enterococcus faecalis, Klebsiella pneumoniae, G. vaginalis, Proteus spp., Acinetobacter baumannii, P. aeruginosa, Streptococcus. spp., Staphylococcus spp., and Candida albicans*) have strong ability to forms biofilms on various artificial surfaces (polyethylene, stainless steel, polypropylene, glass, rubber, wood) (Muhammad et al., 2020; Schulze et al., 2021), which are commonly used in herbal drug industry.

Biofilm production is a cyclical process in various stages and in a progressive manner. The process begins when single planktonic cells adhere to the medicinal plant surface. Several developmental phases can be identified as reversible attachment, irreversible attachment and maturation of biofilm cells (maturation-I and II stages). During reversible attachment, bacteria attach to the substratum via the cell pole or flagellum (step 1), followed by longitudinal attachment. A decrease in flagella reversal rates, flagella gene expression, and the synthesis of biofilm matrix components accompany the transition to the irreversible attachment. This stage is further distinguished by attached cells that exhibit tolerance against harsh environments. Biofilm maturation stages are distinguished by the emergence of cell clusters that are several cells thick and embedded in the three-dimensional biofilm matrix (maturation-I stage) before fully maturing into microcolonies (maturation-II stage). Dispersion has been linked to decreased and degraded matrix components with motile scattered cells that are more susceptible than biofilm cells (Ahmad et al., 2023). An overview of biofilm stages on the surface of medicinal plants is depicted in Fig. 2.

6. Microbial quality framework of herbal drugs

The WHO has suggested a method for detecting the total microbial number in medicinal plant materials (https://www.who.int/docs/default-source/medicines), the most commonly used and globally accepted. In the protocol recommended by WHO, 10 g of samples (plant materials) should be suspended in 90 ml of sterile normal saline solution in double distilled water or sodium chloride-peptone with a normal physiological pH of 7.0. For total aerobic bacterial enumeration, 100 µl of sample should be spread in triplicate on Casein Soybean Digest Agar, Nutrient Agar, or Luria Bertani Agar using standard methodology. Afterwards, spreaded plates should be kept in a bacteriological incubator

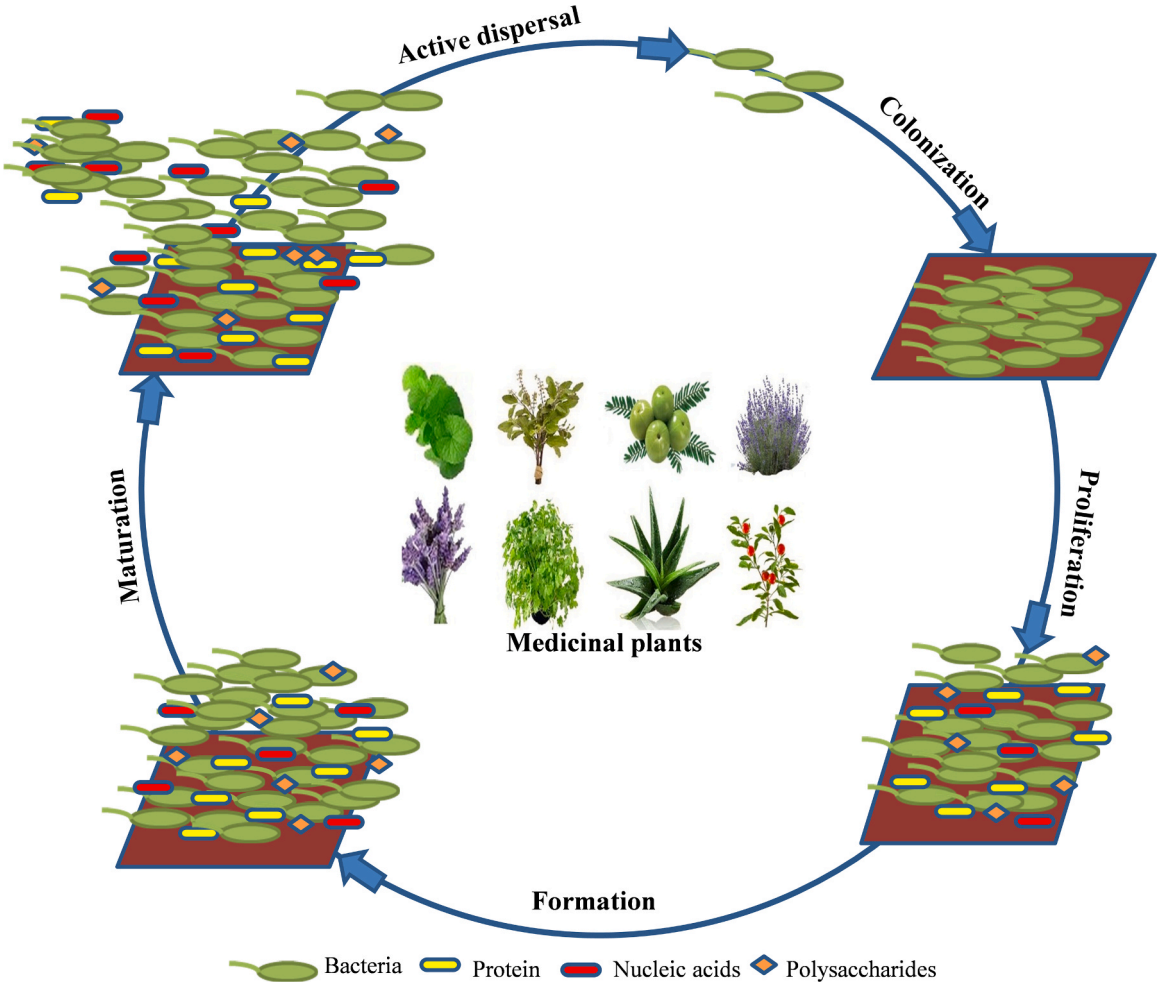

Fig. 2. Model of bacterial biofilm development on the surface of medicinal plants.

at 30–35 °C for 24–48 h for proper bacterial growth. For the enumeration of fungi (yeast and molds), the same suspension of samples should be spread in triplicate on Sabouraud Dextrose Agar or Potato Dextrose Agar with the addition of 10% tartaric acid solution to obtain acidic pH (3.0–3.5). Following the spreading, all the tested plates should be incubated at 20–25 °C for five days to visualize the fungal growth on the plates (Liu et al., 2002). Examination of specific objectionable bacterial pathogens from Gram-negative and Enterobacteriaceae members (*E. coli, Shigella, salmonella, Klebsiella, S. aureus,* and *P. aeruginosa*) consist of specific techniques for isolation, cultivation, and identification through morphological, biochemical, serological and molecular-based approaches. The standard guideline of WHO for total aerobic bacterial pathogens should not exceed more than 10^7 CFU/g of medicinal plant materials (teas and infusion) and below the 10^5 CFU/g for internal application is highly recommended. The WHO specification for fungal pathogens (yeast and mold) should be below or equal to 10^4 CFU/g of medicinal plant materials for infusion and teas and 10^3 CFU/g for internal application. The maximum load of fungi in the herbal plant materials is a great concern due to the feasibility of synthesizing mycotoxins (Aflatoxin), which are carcinogenic in nature. The WHO also recommends a specific test to detect the potential occurrence of aflatoxins, which are very harmful contaminants in any kind of medicinally important plant materials (WHO, 2008). Further, we can directly detect the causal fungi for aflatoxin production on culture plates (Aspergillus Differentiation Medium Base) with the addition of an antibiotic (Chloramphenicol) as a selection marker. Nowadays, microbiologists use sophisticated tools such as Gas chromatography-mass spectrometry, Liquid chromatography-mass spectrometry, and High-performance liquid chromatography (Narváez et al., 2020; De Girolamo et al., 2022) to identify and quantify such neurotoxic and carcinogenic mycotoxins in the medicinal plants.

Despite the high consumption of plant-derived products in many continents, the herbal products market frequently does not follow any quality control standard (Ekor, 2013). Under the safety monitoring of herbal medicines in pharmacovigilance systems, the WHO regulated the registration of herbal medicines and products for commercial use in 2004. Concurrently, the WHO also directed and reaffirmed that all herbal drugs must be evaluated for quality, safety, efficacy, and shelf life prior to registration (WHO, 2008). Another recommendation by the WHO's pharmacovigilance system states that herbal drugs must adhere to pharmacopoeial standards when analyzing herbal products for microbial contamination. Four pharmacopeia (Indian; https://www.ipc.gov.in, USA; https://www.usp.org, European; https://www.edqm.eu and Brazilian; https://www.gov.br/pharmacopeia) have drawn almost standards for herbal medicines for oral application as following: less than 1×10^4 aerobic bacteria/g or ml and 10^2 fungi/g and absence of *Shigella* spp., *Salmonella* spp., *E. coli*, *S. aureus*, and *P. aeruginosa.* Pharmacopoeia also indicates that additional microbial indicators (*Enterobacter* spp, *B. cereus*, *P. aeruginosa*, *C. albicans*, *A. parasiticus* and *A. flavus*) should be absent, to limit the risk of oral application of herbal drugs (Table 2).

In India and European continents, the evaluation of microbiological contamination of medicinally important plants has grown to be a vital part of the concepts of Hazard Analysis and Critical Control Point (HACCP; https://www.fda.gov/food/haccp) and Good Agricultural Practice (GAP; https://apps.who.int/iris). European pharmacopeia recommends that the permissible limit of microbiological contamination for herbal products be 1×10^7 CFU/g for total aerobic bacteria, and 10^5/g for yeast and molds. While, the permissible limit of microbial contamination for herbal products that are used without boiling water is further limited to 10^5 CFU/g for total aerobic bacteria, 10^4/g for fungi, 10^3 CFU/g for Enterobacteria and other Gram-negative bacteria such as *Salmonella* spp. and *E. coli* should be absent. In the context of Gram-positive bacterial contamination to herbal drugs, there are two well-reported Gram-positive phytopathogenic genera including Bacilli (*B. cereus*, *B. anthracis* and *B. thuringiensis*) and Staphylococci (*S. aureus*, *S. hominis*, *S. cohnii*, *S. simulans*, *S. warneri* and *S. lugdunensis*) known for their pathogenic potential and contaminate herbal drugs (de Sousa Lima et al., 2020). Generally, various tests are applied to confirm the presence of microbial contaminants in plant-derived drugs/other products. The microbiological limit shows no significant change and complies with accepted standards for non-sterile natural products (Table 2).

6.1. *Effect of drying on microbial contaminants*

Drying is a crucial step in lowering the plant's moisture content, which reduces microbial and enzymatic activity and extends the shelf-life of herbal drugs (Muller and Heindl, 2006; Thamkaew et al., 2021). Due to its simplicity and capacity to immediately maintain the therapeutic attributes of plant material sustainably, drying is the most broadly used and essential technique for post-harvest preservation of medicinal plants. Additionally, this procedure helps to make it easier to market medicinal plant samples because drying reduces the volume and weight of the plants, making them easier to store and transport to different locations (Catania et al., 2020; Thamkaew et al., 2021).

The drying process enormously improves the microbiological quality of the medicinal herbs by reducing the bacterial and fungal load, respectively (Catania et al., 2020). The selection of drying conditions depends on the moisture content in the harvested tissue, plant components utilized, and the temperature that preserves the essential ingredients of the herbal drugs while restricting the growth and development of microbial contaminants. The drying process lowers the foods' water activity and moisture content, substantially reducing or postponing microbes' growth. Additionally, this technique minimizes the proliferation of pathogenic bacteria and fungi in herbal products (Catania et al., 2020). In order to quickly reduce the microbial contaminants and moisture content of air without compromising the quality of the medicinal plant's bioactive compounds, suitable and sophisticated dryers are required. These suitable dryers should use temperature (35 °C to 46 °C), humidity (22–27%), and velocity ($m \cdot s^{-1}$) throughout the process to minimize microbial interference (Thakur et al., 2011; Catania et al., 2020). Medicinally important plants can be dehydrated and dried in multiple ways, including open air dehydration (shaded from direct sunlight), wire-screened rooms or buildings, placed in thin layers on drying frames, in drying rooms/ovens and solar dryers, microwave or infrared devices, by indirect fire (baking) and lyophilization technique (Bhatta et al., 2020). To prevent microbial spoilage and active chemical ingredients of medicinal plants and herbal drugs, humidity, and temperature should be maintained under control conditions (Roshanak et al., 2016; Thamkaew et al., 2021).

The spray-drying approach has been frequently used to obtain dried extracts with improved technical properties and higher concentrations of biologically active ingredients (Thamkaew et al., 2021). In the spray drying process, a slurry, solution, or emulsion comprising one or more components of the intended herbal product is atomized into droplets by spraying. Then, the droplets are quickly evaporated into solid powder by hot air at a specific temperature and pressure. In this process, less than a minute passes between the medications that need to be dried and the hot air, which is incredibly quick and potentially insufficient to kill the germs. However, the pharmaceutical industries frequently use this method (Al-Zoubi et al., 2021). In a comparative microbiological analysis of drug pulverization, liquid phase extraction and spray drying extraction using the medicinal plant *Phyllanthus niruri* L., liquid phase extraction significantly reduces the microbial load. In contrast, the spray dryer, despite its high temperature, had little impact (Giribabu et al., 2014).

The microbial burden of medicinal plants can be significantly reduced by routine exposure to warm-air ovens and microwaves. However, these techniques are not suggested for medicinal herbs that contain volatile oils. One of the researchers examined the two ways for drying plants and found a reduction in microbial burden (microbial load) on the plant's components. However, warm drying air showed that

most of the volatile compounds were lost at temperatures above 60°C, whereas microwave drying significantly impacted the volatile oil profile.

Other techniques, such as lyophilization (freeze-drying), tray drying, and oven drying, have been previously applied to preserve and protect medicinal plants against microbial contaminants. To date, little research has shown how different drying conditions affect the decline in microbial loads and their colonization in different parts of medicinal plants (Patel and Pikal, 2011; Bhatta et al., 2020). After completion of the drying process, herbal products are packaged in containers for shipping and transport or other further processing is required to ensure the product's efficacy and shelf-life.

6.2. Effect of extraction techniques on microbial contaminants

Medicinal plants can be consumed as tinctures (plants in alcoholic solutions) or less frequently as teas (plants soaked in hot water), or they can be inhaled through steam from boiling suspensions (Zhang et al., 2021). Additionally, plants' dried parts can be applied externally as suspension of petroleum and jelly or as poultices of concentrated teas or tinctures (Romanovskiy et al., 2001; Zhang et al., 2021). These preparations, commonly known as medicinal teas, are made from naturally occurring herbs that are harvested, dried, and packaged without effective sanitary and hygienic control. Therefore, microbiological contaminations are possible in aqueous extracts (de Freitas Araújo and Bauab, 2012; Chandrasekara and Shahidi, 2018), indicating that reducing potential contaminants in the herbal drug preparation industry is crucial. In particular, herbal drugs that are exposed to herbal maceration (cold water extraction) may include a considerable number of microbial populations that could proliferate during maceration (Kumar et al., 2021).

On the other hand, the use of boiling water can reduce viable counts of microbial contaminants and can inactivate potential pathogens (Chandrasekara and Shahidi, 2018). However, the application of hot water can stimulate bacterial spores of the family *Bacillaceae,* which are highly resistant to heat treatment that is frequently applied in the preparation of infusion, and consequently, this heat shock may stimulate spore germination (Kumadoh et al., 2022). Therefore, the extractions employing water (cold and hot) increase the risk of pathogen proliferation. This microbial contamination compromises the plant materials' and products' integrity and quality (Kumadoh et al., 2022). Thus, the selection of the extraction solvent is a crucial factor in preventing microbiological contamination, and the frequently used solvents for extracting active phytochemicals include methanol, ethanol, diethyl ether, hexane, chloroform, acetone, dichloromethane and others (Truong et al., 2019; Abubakar and Haque, 2020). In general, ethanol or methanol-based herbal extraction should offer hygienic conditions, although the outcome may vary depending on the concentration of alcohol used (Truong et al., 2019).

6.3. Influence of pH on microbial contaminants

The pH level is considered one of the key factors influencing the shelf-life and quality of herbal drugs (Pokharkar et al., 2022). The pH regulates a variety of chemical and microbial functions during the preparation and storage of the drugs, such as methanogenesis, syntrophic oxidation, sulfate reduction, iron reduction, and kinetics and thermodynamics of microbial respiration, cell structural integrity, and cell metabolism (Jin and Kirk, 2018). The bacterial count may be low when there is an acidic pH, while high contamination levels in herbal drugs are possible at neutral or basic pH levels (Jin and Kirk, 2018). On the other hand, low pH can support the growth of fungal contamination in herbal drugs (Pokharkar et al., 2022), suggesting that pH affects the type of the most possible contaminant in herbal drugs. This is consistent with the finding that bacterial growth and multiplication are optimal at neutral or between 5 and 8.5 pH levels. On the other hand, a pH level between 8.4 and 9.6 was found to be best suited for fungal proliferation in natural drugs, respectively (Cornet and Gaillardin, 2014; Pokharkar et al., 2022).

6.4. Effect of ozone injection on microbial contaminants

The USFDA (United States Food and Drug Administration) approved the application of ozone as a powerful oxidizer for decontamination and food processing in 2001 in both water-soluble and gaseous forms. The food industry has extended the use of ozone as a safe approach to inactivate bacteria, particularly for fluid foods. The oxidation of double-bond cellular components, such as sulfhydryl groups and phenolic rings, is thought to cause ozone's antibacterial properties, ultimately resulting in cell death (Pandiselvam et al., 2020). Several factors, such as the number of microbial contaminations, nature of microorganisms, pH of the medium, temperature, additives, relative humidity, and the volume of organic matter surrounding the cell, determine how effectively contaminants are removed using ozone technology (Manousaridis et al., 2005). The process of treating water with ozone starts with producing ozone in an ozone generator. After that, ozone is introduced into the water, which causes impurities like metals, germs, and viruses to oxidize and disappear instantly. The organic substance found in the membranes of bacteria, viruses, and parasites is oxidized by ozone. It is essential first to comprehend how ozone is formed to understand the process better. Oxygen is the mother of ozone. There are two oxygen atoms (O^2) in an oxygen molecule and three oxygen atoms (O^3) in an ozone molecule. The energy of electricity and UV light causes oxygen molecules in the air to split into two oxygen atoms. The loose oxygen atoms then recombine with ordinary oxygen molecules to form ozone. In the upper atmosphere, sunlight interacts with oxygen to produce the Earth's protective ozone layer. Ozone is produced nearer the surface when lightning strikes and electricity passes through air that is high in oxygen. Both processes are mimicked in electrical ozone generators and ultraviolet, which make ozone water treatment possible (Manousaridis et al., 2005; Pandiselvam et al., 2020).

The ozone's inactivation strategies against microbes comprise ozone penetrating cells, targeting components of cell membranes, inactivating enzymes, and breaking down the genetic elements of total RNA and gDNA. These modes of action eventually cause cell lysis and leakage of cellular contents and cell lysis of microbial contaminants of herbal drugs.

However, this method's primary disadvantage is the latent toxicity of ozone molecules to the user. Consequently, the decontamination procedure should only be carried out in a separate room with good ventilation. Additionally, ozone should be given time to break down into oxygen molecules, which typically consume 20–50 min at ambient temperature (Thanushree et al., 2019).

6.5. Influence of cold plasma on microbial contaminants

Plasma has been utilized to inactivate bacteria since the mid-1990 s. However, this strategy has recently been considered in the food industry sector. Generally, there are two plasma types: cold and heat. A comparatively ionized gas known as "cold plasma" is created when energy sources, including dielectric barrier discharges (DBDs), corona discharges, microwave discharges, pulse discharges, and high-frequency discharges are coupled with gaseous phases like oxygen, nitrogen, hydrogen, air, argon, halogen, or a mixture of these gases (Sakudo et al., 2020). It generates higher-kinetic-energy collisions between plasma particles like electrons, free radicals, and ions. The plasma system has no net charge. Various factors, including the amount of gas supplied, the surrounding phase, humidity, power, and voltage levels, cause variations in plasma. Reactions of numerous plasma components, such as free radicals, charged particles, ultraviolet photons, ions, and heat, cause microbial cell membrane oxidation and DNA modification, which results in microbe inactivation. Cold plasma is a rapid, ecologically safe, and

low-temperature processing procedure that effectively inactivates microbes in herbal plants.

When reactive species generated by plasma come into contact with contaminated food surfaces in microbial inactivation, electrostatic forces build up at the site of maximum energy. Cell lysis results from much more intense bombardment activity brought on by the energy flow. Heavy bombardment causes surface damage that prevents the pathogenic bacterial cell from quickly repairing itself, ultimately leading to cell death. It is called "plasma etching" in this context. By denaturing chemical bonds and DNA, plasma etching facilitates the cell's antimicrobial properties (Sakudo et al., 2020). Microorganisms are rendered inactive by the oxidation of their cell membranes and DNA, caused by reactions between different plasma components like free radicals, ultraviolet photons, charged particles, heat, and ions (Lee et al., 2015). It has proven effective to use cold plasma, a low-temperature, reasonably quick, and environmentally secure processing technique, to inactivate the microbes of medicinally important plants (Pankaj and Keener, 2018). The effectiveness of plasma decontamination is primarily influenced by the kind of gas, gas composition, voltage, energy source, treatment period, and relative humidity product type (Li and Farid, 2016). The diagrammatic representation pertaining to mechanism of action of cold plasma in decontaminating the medicinal herbs and microbial pathogens are illustrated Fig. 3. However, cold plasma has drawbacks such as limited drug and food penetration capacity (particularly into solid drugs and foods) and commercial availability. Additionally, little evidence is available on its effect on the quality and quantity of the product's active ingredients (Ebadi et al., 2019).

6.6. *Effect of medicinal plant storage on microbial contaminants*

Pre-storage processing of medicinal plant materials includes heat, drying, cooling, lyophilizing, and packaging, and they are applied to minimize the microbial load in medicinal plants (Yu et al., 2020). These treatments can reduce the deterioration of herbal plant materials by preventing microbial contamination (Tanko et al., 2005; Thakur et al., 2011). Medicinal plant storage is crucial in the production process to enhance medicinal value. During storage, gradual changes in several biotic and abiotic factors (physical, chemical, and biological) occur as a result of the components in the outside environment and their interactions with their own physical and chemical attributes. Due to the heat exchange capability of poorly ventilated storehouses, prolonged storage of medicinal herbs characteristically results in a rise in sample moisture content, rendering medicinal herbs more susceptible to fungal growth and mycotoxin synthesis. The abundance of microbial contaminants in medicinal plants is fungi. However, most of these fungal communities are likely to be observed as commensal inhabitants of the medicinal plant that endured drying and pre-storage limitations. Most fungi are found on medicinal plants; if relative humidity is not maintained during storage, they can grow after harvest too and deteriorate the plant materials expressively (de Sousa Lima et al., 2020; Yu et al., 2020).

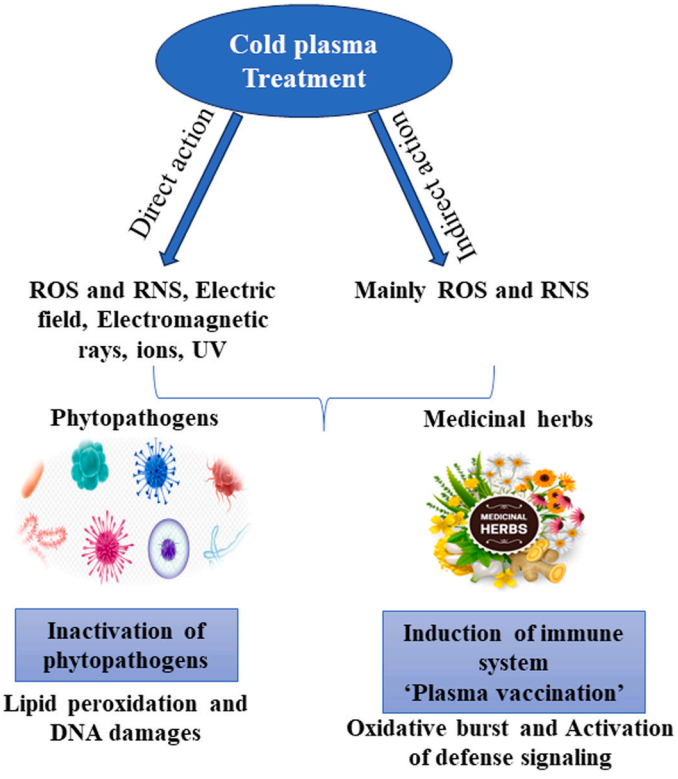

Fig. 3. Diagrammatic representation pertaining to mechanism of action of cold plasma in decontaminating the medicinal herbs and microbial pathogens.

The degradation of some basic ingredients and essential compounds of several medicinal plants can be ascribed to molds belonging to *Aspergillus* spp. and *Penicillium* spp. (de Sousa Lima et al., 2020). These fungal contaminations may reduce the quality of raw herbal drugs in terms of the content of active compounds, negatively affecting the therapeutic efficacy and the market value (Kumar et al., 2009; Rajeshwari and Raveesha, 2016). For example, twenty-eight fungal species were recovered and identified from samples of herbal parts of *Codcnopsitis pilosulas, Rhizoma glycyrrhizae, Radix notoginseng, Armeniacae amarae, Magnoliae officinalis, Fritillariae cirrhosae* and *Lonicerae japonicae* stored for sale in markets of India, Ibadan, and Nigeria for mycoflora associated with their storage was examined, demonstrating that some herbal medicinal plants pose very hazardous to people's health (Sofowora et al., 2013; Okaiyeto and Oguntibeju, 2021). Numerous investigations have reported that Aflatoxin is used in several raw herbal materials. One of the research workers examined 152 dried medicinal and aromatic plants used as raw pharmaceutical materials to assess the prevalence of toxigenic fungi and associated mycotoxins in appropriate storage conditions in Argentina. This study observed that *A. parasiticus* and *A. flavus* were most prevalent, and high aflatoxin concentrations were detected. Mycotoxin contamination is a possible hazard, particularly during extended storage in unsanitary settings without moisture, temperature, and control, which often render medicinal plants more prone to mold proliferation and mycotoxin synthesis (Altyn and Twarużek, 2020).

The drying process is applied to reduce the activity of plant enzymes and inactivate microorganisms in the natural drug system. Due to their propensity for being hygroscopic (quickly absorbing moisture), dried plant materials must be stored under controlled humidity and appropriate temperature. Rehydration may lead to the breakdown of bioactive compounds by microbial enzymes and plants themselves. Substantial bacterial and fungal contamination suggests inadequate storage facilities and poor hygiene practices during herbal drug preparation. It is vital to prevent additional contamination throughout the storage processes of these medicinal products to ensure the medicinal value of herbal plants.

Regarding quality maintenance of herbal drugs, bio-deterioration of these products by associated harmful fungi during storage has gained wide attention to ensure shelf-life (Singh et al., 2022). Investigation on the long-term shelf-life of dried herbal products and preparations is still under exploration. For example, the degradation of several herbal drugs (cinchona bark, willow bark, opium poppy, and foxglove, turmeric, Devil's claw and Ginseng)) that were collected and stored by traders for 6–9 months was investigated that some of the contaminated herbal products have deteriorated by toxigenic strains of *A. flavus* that synthesize aflatoxin B1 which was beyond to permissible limit (Ekor, 2013). Dried leaves of *Plantago lanceolata* were subjected for 24 weeks to atmospheres with varying relative humidity (75%, 45%, and 0%), and chemical alterations of compound of interest were assessed,

demonstrating the loss of bioactive compounds (aucubin, catalpol and acteoside) with colonizing pathogenic fungi (*Aspergillus parasiticus*, *Aspergillus flavus* and *Penicillium* spp.) (Gonda et al., 2012).

It is widespread practice for herbalists to formulate herbal medicines and store them in a refrigerator for further applications. However, a previous study examined how microbial contaminants impair the beneficial ingredients in African plant extracts (Gingerol, Artemisinin, Curcumin Bisdethoxy curcumin, and kolaviron) reported by Fenibo et al. (2019) and Abodunrin et al. (2022). According to this study, there may be few or no active compounds left after 25 days of low-temperature storage, primarily because of spontaneous breakdown by microbial communities prevalent in nature (Fenibo et al., 2019; Abodunrin et al., 2022). The World Health Organization (WHO, 2007) strongly suggests that fresh medicinal plant materials should be stored at acceptable low temperatures, ideally between 2 and 8°C. At the same time, frozen items should be stored at or below −20°C (Abodunrin et al., 2022). The benefits and disadvantages of all the above discussed techniques is listed in Table 3.

Table 3
Listed benefits and disadvantages of various decontamination techniques.

Decontamination techniques	Benefits	Disadvantages
Drying	Quick and safe conservation of the medicinal qualities of the plant material in an uncomplicated manner. Removes the moisture from the food so that bacteria, yeasts, and molds cannot grow and spoil herbal products. Slows down the action of enzymes but does not inactivate them.	Hot-air drying could lead to major degradation of herb's aroma. High drying temperature could lead to the degradation of pigments. It can take several days to complete.
Extractions methods	The extraction of phytoconstituents at low temperature strictly avoids damage from heat and some organic solvents. No solvent residues. Environmentally friendly extraction procedure.	Prolonged extraction time, substantial amounts of solvents, and at times many extraction steps. Significant amounts of thermolabile phytochemicals turn out to be either decomposed or degraded during heating.
Ozone injection	The treatment process occurs without adding any kind of chemicals to the water. Ozone is effective over a wide pH range and rapidly reacts with bacteria, viruses, and protozoans and has stronger germicidal properties than chlorination.	The treatment process occurs without adding any kind of chemicals to the water. Ozonation provides no germicidal or disinfection residual to inhibit or prevent regrowth of bacteria.
Cold plasma treatment	Low temperature treatment. Plasma generation in ambient air. Low operating cost. Short treatment time. Environmentally friendly. Selective effect.	Required large number of samples. Investment cost. Adaptation mechanisms. Determination of effective dose. Depth of plasma penetration.
Storage condition	It enhances the stability of crude herbal drugs by excluding the direct impacts of oxygen, light, microbes and insects on crude drugs.	Herbal products often suffer degradation during storage by oxidation, hydrolysis, crystallization, emulsion breakdown, enzymatic deterioration and chemical reactions with the additives and excipients.

7. Novel approaches for decontamination of medicinal plant materials

Decontaminating and preserving these therapeutic plants and their derivative products have always been a goal in the quest for safer, more natural, and more effective remedies. Numerous decontamination techniques, including fumigation and UV radiation, have been tested to decontaminate the plant parts since heat treatments are not allowed due to the volatile nature and heat sensitivity of several key active components of herbal drugs (Rahmati et al., 2022). However, the UV irradiation approach was inappropriate due to its low penetrating power on plant materials (Balakrishnan et al., 2021). Although fumigation approaches using gaseous ethylene oxide to reduce the microbial load in herbal samples, they are currently banned in many nations since one of its residues (namely ethylene chlorohydrin and ethylene glycol) is highly carcinogenic (Duncan et al., 2017). Disinfectant techniques have been proposed to safely decontaminate medicinal plant parts, such as electromagnetic radiations, ultrahigh pressure, photodynamic pulsing, and CO_2 treatment (Balakrishnan et al., 2021).

Considering certain limitations of the above decontamination techniques, Gamma irradiation, and radiofrequency heating are increasingly gaining attention as a phytosanitary treatment method for herbal drugs/products since they raise hygienic standards and decrease the losses from microbial contamination (Rahmati et al., 2022). This approach rejects the industry's reliance on chemical fumigants and preservatives and is quick, safe, useful, and environmentally friendly in the current era (Suryadi and Mun'im, 2021). This approach removes microbial contamination after packaging the products (de Sousa Lima et al., 2020). In particular, increasing gamma radiation dosage (10KGy) could decline the microbial load significantly below the permissible limit in medicinally important plants (Suryadi and Mun'im, 2021).

Certain medicinally important plants (e.g. *Piper longum, Piper nigrum, Pancratium illyricum, Teclea afzeli, Rauwolfia serpentina, Sanguinaria canadensis, Chelidonium majus, Macleaya cordata, Ipomoea muricata, Holarrhena antidysenterica, Allium sativum, Armoracia rusticana, Diplotaxis harra, Armoracia rusticana, Eutrema japonicum, Alliaria petiolate, Brassica campestris, Brassica rapa, Brassica oleracea, Scutellaria baicalensis, Thymus vulgaris, Scutellaria lateriflora* and *Scutellaria baicalensis*) encompass antimicrobial substances which employ distinctive inhibitory activities on pathogenic microorganisms, such as peptides, oils, liquids, and organic extracts (Vaou et al., 2021). For example, some medicinal plants belonging to *Apiaceae, Asteraceae, Ericaceae, Rosaceae, Ranunculaceae, Zingiberaceae, Piperaceae, Amaranthaceae, Lamiaceae, Apiaceae, Costaceae, Lamiaceae, Malvaceae* and *Rosaceae* encompasses essential oils (menthol, menthone, limonene, α-pinene, camphor, terpinolene and others) with antibacterial activities against mold and mycotoxin-producing fungi (D'agostino et al., 2019). Numerous studies have shown the efficacy of potent plant antimicrobials (Allicin, Berberine, Piperine, Quinoline, Reserpine, Sanguinarine, Tomatidine, Chanoclavine, Squalamine, Ajoene, Isothiocyanates, Sulforaphane, Berteroin, Baicalein, Resveratrol, Kaempferol, Curcumin, Caumarins and Terpenes) in preventing the growth and spread of pathogenic microbial population (*Staphylococcus aureus, Streptococcus pneumoniae, P. aeruginosa, Enterobacteriaceae, Acinetobacter baumannii, Candida albicans, Aspergillus flavus, Aspergillus parasiticus, Stenotrophomonas maltophilia, Mycobacterium smegmatis, Mycobacterium aurum, Staphylococcus epidermidis*, and *Streptococcus agalactiae*) in the tested herbal products (Khameneh et al., 2019; Hemeg et al., 2020; Vaou et al., 2021).

Furthermore, it is crucial to investigate the properties and quality of the bioplastics when they interact with herbal items. Research on the literature has revealed that using biopolymers in packaging herbal medications is entirely restricted. The use of biodegradable and premium polymers, as opposed to conventional wraps, films, labels, and laminates made of fossil fuels, is a significant step in the correct direction for preserving medications and shielding them from microbiological contaminants. It is necessary to conduct further research on this type

of packaging material and added value, such as by introducing smart molecules that can provide information on the nutritional values and properties of the products inside the container. Research on packaging materials is required to improve barrier qualities and guarantee the integrity of the properties of herbal products. The most useful and novel concept for preventing contaminants and extending shelf-life is the active packaging of herbal products. It has been described as a system where the herbal product, the packaging, and the surroundings work together to improve the product's shelf-life or attain certain qualities that would be impossible to create in any other way. Many modern active packaging technologies protect the food from contamination or deterioration while enabling the active ingredient to fulfill its intended function and avoiding direct contact with the herbal product (Ndhlala et al., 2012; Salgado et al., 2021).

8. Conclusions and future perspectives

Microbiological contamination might reduce the efficacy and shelf-life of herbal medicines due to disruption of formulation's quality and stability and altering physical properties and appearance. This could lead to the inactivation of bioactive ingredients and additives in formulations, resulting in a constant loss of the company's reputation. Herbalists should be trained in Good Manufacturing Practices (GMP), safe handling, good harvesting practices, and storing herbal therapeutic products because microbial contamination is a severe problem. It is strongly recommended that more research should be conducted on herbal drugs/products to identify other microbiological contaminants and determine how to decrease them below the permissible limit. The load of microbes and other contaminants should be established, isolated, and identified for further drug safety and shelf-life management. Furthermore, different procedures, such as product treatment with ionic radiation or useful chemicals, may effectively decontaminate microbial contaminants. These novel decontamination techniques might be viewed as a compromise between assuring the product's microbiological safety and minimizing consumer risk. To apply these methods for decontamination, special legal permission/advisory are essentially required. More detailed studies on commonly used medicinal plant species are required to assure quality, efficacy, and shelf-life, which could be vital for providing safety, reliability, and security for its application. Microbial-free herbal drugs should be used to treat diseases in traditional medicine systems as it would reveal better results and gain the trust of patients. It is still very important to conduct adequate studies on the microbiological safety of herbal medicines in the Indian nation. We recommend the establishment of resilient and intensive herbal safety mechanisms in Indian subcontinents to improve efficacy and shelf-life and, therefore, protect public health. Hence, regulatory policies on herbal drug safety and hygiene are required to be standardized and strengthened globally.

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Author contributions

The author FAA conceived the idea and developed the review concept. FAA, FMH, ASK, NZA and RPM collected and surveyed the literature. MP edited the language and composition of the manuscript in its present form. All the authors contributed during the compilation and preparation of review article.

CRediT authorship contribution statement

Fohad Mabood Husain: Writing – review & editing, Data curation. **Asma Sattar Khan:** Supervision. **Noor Zaheer Ahmed:** Supervision, Project administration. **Ram Pratap Meena:** Formal analysis. **Firoz Ahmad Ansari:** Writing – original draft, Resources, Formal analysis, Data curation, Conceptualization. **Michele Perazzolli:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

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Data availability

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2024.100070.

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