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A Comprehensive Overview of Herbal Contaminants and Residues: Regulations and Analytical Methods.

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Abstract: Traditional medicines, often referred to as herbal phytoconstituents, are generally considered safe with minimal negative effects. Herbal remedies are a booming industry, valued at 60 billion dollars worldwide, and are gaining popularity in Western societies. Recent research indicates that many herbal formulations on the market have not undergone thorough quality and efficacy testing. The main source of toxic heavy metals for plants is human activity, including burning fossil fuels and waste incinerators, using leaded gasoline, operating cement plants, processing ore in metallurgical facilities, and discharging sewage sludge without control. Additionally, agricultural chemicals like phosphate fertilizers containing cadmium can contaminate the plants they are used on. Lead, cadmium, mercury (especially in organic form such as methylmercury), and arsenic (only in mineral form) are the primary hazardous metals that pose health risks to humans. These metals do not have any essential or beneficial effects on living organisms. The study suggests that comprehensive quality checks using advanced analytical methods are necessary to ensure the safety of these products. Therefore, adhering to pharmacopeial standards guidelines is important when examining contaminants and their limits in herbal compositions. This review emphasizes the importance of impurity profiling in maintaining the quality of natural products and recommends the use of straightforward, accurate, and precise analytical techniques to eliminate impurities, adulterants and combined synthetic materials in herbal products

Keywords: Herbal contaminants, pesticide residue, Herbal Products, heavy metals, GC-MS, Regulations.

1. Introduction:

Natural herbal compositions have been shown to have high therapeutic potential. These substances are produced by microorganisms, marine creatures, insects, and amphibians through biochemical processes. They are mostly genetically encoded and synthesized by secondary metabolic pathways. Herbal phytoconstituents offer several advantages over manufactured drugs, including greater therapeutic outcomes and fewer side effects for a range of disorders. As a result, their use has increased during the COVID-19 pandemic [1]. Moreover, these herbal products are often more affordable, which improves access to healthcare, especially in regions with limited resources. Additionally, they are known for their superior effectiveness in treating various medical conditions, including their potent anti-inflammatory, antioxidant, and antibacterial properties [2]. Furthermore, the use of herbal phytoconstituents has been associated with a reduced risk of cancer and neurological disorders such as Alzheimer's. Herbal treatments are generally considered safe as they have fewer adverse effects

compared to manufactured pharmaceuticals. In 1991, one in four persons in the UK used herbal remedies, according to global data, indicating a steady increase in the usage of these treatments. In the US, up to 66.6% of people have used complementary and alternative medicine at some point in their lives [3]. It was estimated that one in two Australians used herbal products in 2000. The market for herbal phytoconstituents is expected to expand by 20% annually. In India, sales of medicinal plants have climbed by almost 25% in the last ten years. The commercialization of herbal products has expanded significantly on a global scale due to several important factors [4]. However, there are various issues with their production processes, safety, consistency, and effectiveness [5]. Recent research indicates that many herbal formulations available on the market today have not undergone quality and efficacy testing. Regulatory agencies have shown increased interest in natural and herbal products in recent years due to the documented negative effects of contaminants from various sources.

Heavy metals, which are naturally occurring minerals and ores, are not readily available to living things and thus do not typically pose a health danger to humans. However, they are released into the environment through various natural processes (such as erosion or volcanic activity) as well as human activities (such as cement production, mining, smelting, sewage sludge discharge, refinery emissions, burning of fossil fuels, waste incineration, and use of leaded gasoline). The absorption of heavy metals by plants from the soil can vary significantly. Factors such as the plant's root system and soil characteristics, including pH (higher absorption in acidic soils due to increased solubility of heavy metals), can affect their availability to the plant [6,7]. The amount of lead, cadmium, and other metallic trace elements in raw materials can vary significantly depending on the plant component, habitat, and soil concentration [8–10]. Industrial operations have been shown to cause unusually high accumulations of lead and cadmium in medicinal plants. The need to consider the levels of toxic metals in herbal drugs, their preparations, and their final products has emerged for two main reasons.

A. Environmental contamination with toxic metals significantly increased during the 20th century, particularly due to cadmium emissions. However, lead emissions have decreased in developed countries in recent decades, mainly because of the shift to unleaded petrol [11]. The sources of this pollution are diverse, including industrial activities, use of purification mud, and agricultural practices such as the use of cadmium-containing phosphate fertilizers, mercury fungicides, and arsenical insecticides. These agricultural treatments were heavily used in the past and are still used in some countries today [12]. It is widely recognized that the main source of cadmium entering soil and the food chain is through the use of fertilizers. The SCTEE provided an opinion on the accumulation of cadmium in agricultural soils due to the use of fertilizers [13]. For example, the roots of valerian grown in soil treated with sewage sludge contained significantly higher levels of cadmium, lead, and mercury compared to roots grown in soil without any amendments [14].

B. Several reports have indicated that exotic herbal treatments, particularly those from Asia, may contain harmful levels of arsenic and/or heavy metals.

2. Toxic chemical Sources in herbal products:

Heavy metals from land, water, and air can easily contaminate medicinal plants. Point sources, such as various industrial operations, often release these metals into the atmosphere, which can then settle in the soil. Additionally, rainfall, airborne dust, and plant protection agents can further contribute to

the contamination of plants. Wastewater contains toxic substances that can pollute the environment, water sources, agricultural soils, and ultimately the human food chain. Contamination leads to crops absorbing undesirable concentrations of metallic elements. The main factors influencing metal uptake by plant roots include soil characteristics, metal properties, and the type of plant [15]. Therefore, understanding metal mobility within plants is essential for evaluating how soil pollution impacts the absorption of metals by plants. Increased levels of heavy metals in plants have been associated with locations near busy roadways, areas exposed to long-term treated or untreated wastewater, and former landfill sites. These heavy metals can enter the human body when the plants are harvested and processed into dosage forms. This accumulation may lead to health issues, including hypertension, stomach discomfort, skin rashes, intestinal ulcers, and various types of cancer, affecting the liver, lungs, heart, kidneys, brain, and central nervous system. [16]. Moreover, herbal contamination often occurs due to poor storage and transit conditions, which can result in the loss of active components, the formation of inactive metabolites, and the creation of harmful metabolites. Additionally, herbal medications can be compromised during storage by pests such as mites, nematode worms, insects, and beetles. Pesticide residues from agricultural practices, such as spraying, soil management during farming, and fumigant administration during storage, are known to be present in herbal medications [17].

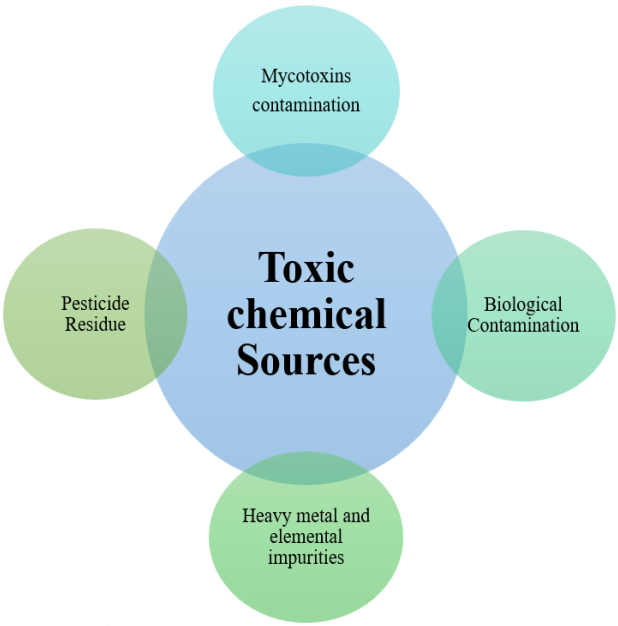


Fig.1 Toxic chemical Sources in herbal products

Table 1. Industrial uses, toxic effects and limits of some heavy metals

Heavy Metal	Industrial uses	Toxic effects	Permissible limits (mg/l)
Arsenic	Pesticides, herbicides	Lung cancer, skin problems	0.02

Cadmium	Plastics, Pigments, Batteries, Plating	Lung cancer, Bone disorder, Kidney damage	0.06
Chromium	Dyes, Alloys, Tanning	Kidney and liver damage, dermatitis, Respiratory effects	0.05
Lead	Batteries, Wire and cable, Alloys	Neurological effects, Hematopoietic system damage and reproductive effects	0.1
Mercury	Chloro alkali industry, Pesticides, thermometers, Batteries	Kidney damage and Neurological effects	0.01
Manganese	Pesticides, Batteries	CNS effects	0.26
Zinc	Pharmaceuticals, Dyes, Batteries	Gastrointestinal disturbance and Anemia	15

2.1. Biological Contamination:

Impurities in medicinal plants, along with their preparations and products, are known as biological contamination. Live microorganisms, such as bacteria (including their spores), yeasts, molds, viruses, protozoa, and insects (including their eggs and larvae), can contribute to contamination [18]. Chemical pollutants, on the other hand, are byproducts of microbial metabolism and may include harmful low-molecular-weight substances produced by molds. Herbs and their products can become contaminated by microorganisms due to improper handling during production and packaging. The most common sources of contamination are microorganisms found in soil and processing facilities, polluted air, and those generated by humans. Additionally, cross-contamination can occur when foreign materials, such as glass, plastics, and other substances, come into contact with herbal preparations, medicinal plants, or products [19,20]. Feces from animals and humans, as well as animal dung, can all contribute to biological pollution. According to the World Health Organization (WHO), quality assurance measures such as Good Manufacturing Practices (GMP) for herbal medicines and Good Agricultural and Collection Practices (GACP) for medicinal plants should be implemented to prevent and manage contamination. Currently, few medicinal plants are gathered from the wild, and there is limited data available to evaluate the biological contamination of both wild and cultivated medicinal herbs. The primary objective of standards such as GACP (Good Agricultural and Collection Practices) and GMP (Good Manufacturing Practices) is to reduce the overall risk of contamination, addressing not only biological issues but also other potential concerns. [21].

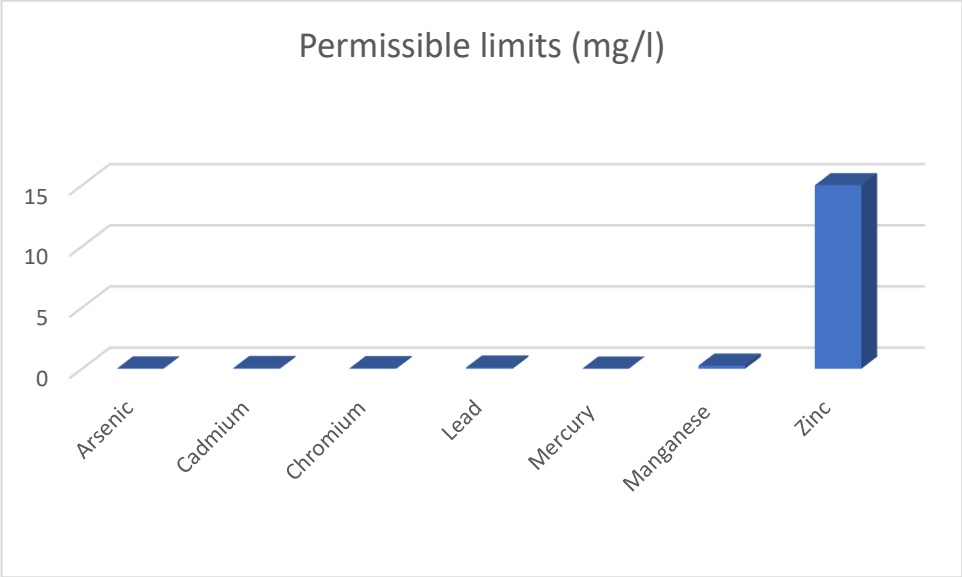
The pharmacopoeia has published studies on the microbiological standards for herbal remedies, specifically focusing on the absence of certain harmful bacteria. Key indicators of microbiological

quality include the absence of Salmonella, low levels of E. coli, and the absence of bile-tolerant Gram-negative bacteria. Additionally, the total aerobic microbial count and the total yeast and mold count are also important indicators [22]. These are typically represented in terms of colony-forming units per gram (CFU g^{-1}) or per milliliter (CFU mL^{-1}) of raw herbal materials or dosage forms. The limitations for various microbiological quality categories of herbal medical products are outlined in Table 2. Products classified as Category A include herbal medications, with or without excipients, that are intended to be prepared as decoctions or infusions using boiling water [23]. This category also encompasses traditional tea brewing. Extracts and herbal medicines that have undergone pre-treatment to reduce microbial contamination are classified as Category B. If the pre-treatment, such as extraction or processing with non-boiling water or low-strength alcohol, fails to meet the decontamination standards of Category B, the product is categorized as Category C. A significant health risk arises when herbal products and medicinal plants are contaminated with bacterial strains that are resistant to known antibiotics [24]. In a study conducted by Brown and Jiang, they analyzed 29 herbal supplements purchased from local stores in the United States for the presence of antibiotic-resistant bacteria. They identified several resistant organisms, including Bacillus, Erwinia, Ewingella americana, Staphylococcus, Enterobacter cloacae, and Stenotrophomonas maltophilia [25]. The study revealed that antibiotic resistance was notably prevalent for ampicillin, nalidixic acid, trimethoprim, ceftriaxone, and streptomycin. Additionally, teas containing opportunistic microbial species, such as molds and bacteria, can pose a risk to patients, particularly those who are immunocompromised, such as individuals suffering from AIDS [26].

The microbiological quality of commercially available tea bags, including those containing fennel, chamomile, peppermint, and various fruit teas, was evaluated by Wilson et al. in their study [27]. According to the manufacturer's recommendations, some tea bags were steeped in water at 90 °C for five minutes, while others were soaked in water at 67 °C from an automated coffee maker's hot water outlet in a bone marrow transplantation hospital. At 90 °C, non-fermentative Gram-negative bacteria were found in all tea infusions studied; however, spore-forming bacteria were more prevalent in the tea bags containing peppermint and chamomile. The high temperature of 90 °C significantly reduced the amount of mold in the teas. A study by Wilson et al. found that fennel and chamomile teas dramatically promoted the growth of several bacterial species, including A. baumannii, E. coli, E. faecalis, and P. aeruginosa. After 6 hours, the concentration of A. baumannii in chamomile tea increased from 103 CFU mL^{-1} to 105 CFU mL^{-1}. After 24 hours of preparation, this concentration rose even further to 108 CFU mL^{-1}. In contrast, the levels of E. coli showed minimal change during the same 24-hour period. Peppermint tea displayed similar results, except for the E. coli count. Additionally, the count of S. aureus steadily decreased from 1.1 x 103 CFU mL^{-1} to below 27 CFU mL^{-1} over the following eighteen hours [27]. This indicates that the teas served in the hospital were conducive to the growth of microorganisms.

Medicinal plants may also harbour Clostridium spores. In the analysis conducted by Martins et al., it was found that 83.9% of the herbal samples contained Clostridium perfringens spores. However, only 19.2% of these samples had concentrations exceeding 10^3 spores per gram, indicating a notable level of contamination. Among the thirteen chamomile samples examined, eight were contaminated with Clostridium botulinum spores. Additionally, linden tree blooms (Tilia platyphyllos Scop.), orange tree

leaves (Citrus sinensis (L.) Osbeck), and maize silk (Zea mays L.) were also found to have high levels of Clostridium perfringens spores. Interestingly, Bacillus cereus spores, which can also cause foodborne illnesses, were detected in 96.8% of the samples, with more than 10^3 spores per gram present in 60% of the samples [28]. There is limited evidence that serious microbiological illnesses, such as those caused by Shigella, Salmonella, Listeria monocytogenes, and Vibrio, are contaminating therapeutic plants. However, between October 2002 and July 2003, infants in Germany under the age of 13 months were infected with Salmonella enterica serovar Agona. The outbreak was linked to contaminated herbal tea bags that contained aniseed (Pimpinella anisum L.), fennel (Foeniculum vulgare Mill.), and caraway (Carum carvi L.). This incident highlighted the critical need for stringent microbiological quality control of medicinal plants and herbal mixtures. [29].



Graph 1. Heavy metals with permissible limits

2.2. Heavy metal and elemental impurities:

The European Pharmacopoeia's general monograph (Ph.Eur. 1433) outlines the specific limits for heavy metals in herbal remedies. The permissible levels are as follows: cadmium at 1.0 ppm, lead at 5.0 ppm, and mercury at 0.1 ppm [30]. Individual monographs for particular herbal medications may provide exemptions with higher limits. It's important to note that "herbal products" are not included under the ICH Q3D guideline concerning elemental impurities, which was adopted into the European Pharmacopoeia at the end of 2016 [31]. However, this guideline requires conducting a risk assessment of the finished product. This assessment should consider potential contamination from excipients, manufacturing equipment, and packaging materials. While the general monograph (Ph.Eur. 1433) remains applicable for assessing the finished herbal pharmaceuticals, a comprehensive risk evaluation of the entire production process is essential.

1. **Cadmium & lead:** Lead and cadmium have been the focus of much research concerning the presence of harmful metals in therapeutic plants. Research has shown that the levels of lead and

cadmium contamination in herbal medicines can vary significantly depending on the type of plant used. A summary of findings published by a German working committee on pollution included a heavy metal database. This database measured the lead and cadmium content in over 12,000 samples, representing 204 different herbal medications. The data included comparisons of the 90th percentile—which indicates the level below which 90% of the results fall—as well as the minimum and maximum values observed [35]. Although significantly fewer raw materials had a 90th percentile lead concentration exceeding 5 mg/kg, several herbal medications had a 90th percentile cadmium level surpassing 0.5 mg/kg and even exceeding 1.0 mg/kg (see Table 1). A market surveillance study was conducted to assess the cadmium content in various herbal medications available in the European market. This investigation was carried out by the Official Medicines Control Laboratories (OMCL) network under the European Directorate for the Quality of Medicines & Healthcare (EDQM) [36]. The main findings are presented with the generous consent of the European Directorate for the Quality of Medicines & Healthcare (EDQM). While significantly fewer raw materials had a 90th percentile lead level exceeding 5 mg/kg, several herbal medications had a 90th percentile cadmium level surpassing 0.5 mg/kg, with some even exceeding 1.0 mg/kg (see Table 1). A market surveillance investigation into the cadmium content of various herbal medications available in the European market was conducted by the EDQM's Official Medicines Control Laboratories (OMCL) network.

2. **Mercury:** There are several forms of mercury present in the environment. These include elemental mercury (Hg(0)), divalent mercury (Hg(II)), which is typically found as mercuric chloride ($HgCl_2$) or mercuric sulfide (HgS), and monovalent mercury (Hg(I)), which is present as mercurous chloride (Hg_2Cl_2). [37]. Additionally, organic mercury occurs when mercury combines with carbon to form compounds. The most common and harmful form of mercury is methylmercury (CH3Hg+), which poses significant health risks (see II Health hazard). Anaerobic sulfate-reducing bacteria are found in aquatic environments such as lakes, rivers, sediments, and the ocean [38]. These bacteria convert divalent inorganic mercury into methylmercury, which is the form of mercury that most readily bioaccumulates in organisms. Methylmercury is biomagnified in aquatic food chains that include bacteria, plankton, herbivorous fish, piscivorous (fish-eating) fish, and macroinvertebrates [39]. As a result, top-level aquatic predators can have methylmercury concentrations that are up to a million times greater than those found in the surrounding water. Arsenic was not detected in samples of herbal medications after examination. Following the testing of 12,000 plant samples, it was concluded that the concentration of arsenic was negligible compared to the threshold value of 0.1 mg/kg [39,40].

2.3 Pesticide Residue:

Chemical substances known as pesticides are utilized to control or eliminate pests. They are categorized as insecticides, fungicides, nematocides, herbicides, rodenticides, and more, based on their function [41]. Pesticides can be classified into several categories based on their chemical composition. These include:

1. Organochlorine pesticides (OCPs), such as dichlorodiphenylethanes (DDT) and hexachlorocyclohexanes (HCH).
2. Pesticide containing nitrogen ex: atrazine and propazine.
3. Plant origin pesticides ex: rotenoids and pyrethroids

4. Organophosphorus pesticides (OPs), such as dichlorvos, malathion, and parathion. Pesticide residues, along with their metabolites and breakdown products, can persist in plants and soil, posing a significant risk of contamination for herbal remedies.

Pyrethroid insecticides have been detected in both native and imported medical products, such as the roots of Panax ginseng and Panax notoginseng. Although these compounds are highly soluble in lipids, the human body is quite effective at breaking them down and eliminating them. Pyrethroids are increasingly replacing organochlorine pesticides (OCPs) and organophosphates (OPs) due to their superior insecticidal properties and lower toxicity to animals [41]. Only a few OCPs, like hexachlorocyclohexane (HCH), and some OPs, such as carbophenothion, have persistent effects (World Health Organization, 2007). [42]

Organochlorines are stimulants of the central nervous system that can lead to symptoms such as tremors, hyperexcitability, and seizures. These pesticides remain in the environment for long periods and accumulate in tissues as they move up the food chain, posing significant risks. While they are often less acutely dangerous than carbamates or organophosphates, their long-lasting presence is concerning. Residues and breakdown products of organochlorine insecticides have been found in soil, vegetation, and animal tissues, spanning from the middle of the Pacific Ocean to the Arctic Circle. Additionally, studies have detected these chemicals in human breast milk. [43,44].

Nervous system symptoms, including headache, dizziness, tingling sensations (paresthesia), tremors, lack of coordination (discoordination), and convulsions, are the main side effects associated with excessive exposure to organophosphates (OPs). This occurs because OPs inhibit the enzyme acetylcholinesterase, preventing the buildup of acetylcholine in nerve tissue and at the effector organs [45]. Consequently, cholinergic synapses remain overstimulated. A primary long-term effect of OP exposure is delayed neuropathy.

2.4 Mycotoxins contamination:

Many fungi produce mycotoxins, which are secondary metabolites that can contaminate a wide variety of food and agricultural products worldwide (CAST, 2003). These mycotoxins can directly contaminate plant materials or products [46], or they can transfer into animal tissues, milk, and eggs after animals consume contaminated feed [46,47]. As a result, mycotoxins in agricultural commodities pose risks to both humans and animals. The Food and Agriculture Organization (FAO) estimates that mycotoxin contamination affects approximately 25% of global agricultural products [48] The mycotoxins of greatest concern for food safety include aflatoxin A (OTA), patulin, aflatoxins B1, B2, G1, G2, M1, and M2, ochratoxin A (OTA), trichothecenes (primarily nivalenol (NIV) and deoxynivalenol (DON)), T-2 and HT-2 toxins, zearalenone (ZEN), and zearalenol (ZOL) (see Table 2). Unsanitary conditions during the planting, harvesting, processing, storage, and transportation of medicinal plants can lead to several issues, including mold development, spoilage, and the formation of mycotoxins. The three primary factors that influence the production of mycotoxins are the fungal strain, plant genotype, and environmental conditions [49,50]. In poorer countries, mycotoxin contamination tends to be more prevalent due to inadequate storage methods and a lack of adherence to Good Agricultural Practices (GAP) and Good Harvesting Practices (GHP).

Medicinal plants, like other agricultural crops, can be contaminated with various mycotoxins. Table 2 presents some of the main mycotoxins found in medicinal plants, along with the plant species that

produce them. The two most significant and prevalent mycotoxins detected in raw medicinal plants are aflatoxins and ochratoxin A. Numerous studies have documented mycotoxin contamination in aromatic herbs and medicinal plants, as well as related products, in several countries, including China [51], India [52], Sri Lanka, Malaysia, and Indonesia [53]

3. Analytical & biomedical screening:

3.1. Chemical Analysis:

3.1.1. Gas Chromatography for volatile constituents:

Gas chromatography (GC) is an essential tool for analyzing volatile substances in herbal remedies. The analysis of volatile oils using GC offers several advantages. First, it provides a distinctive "fingerprint" that can be used to identify the plant species based on its volatile oil composition. Additionally, GC makes it easy to detect contaminants in the volatile oil, and the specific composition and relative concentrations of organic components are unique to each plant. Second, the components can be accurately identified through GC coupled with mass spectrometry (GC-MS), and the extraction process for volatile oils is straightforward and standardized. Furthermore, the relative amounts of various constituents in the oils can help track or evaluate certain properties of the herbal medications [54].

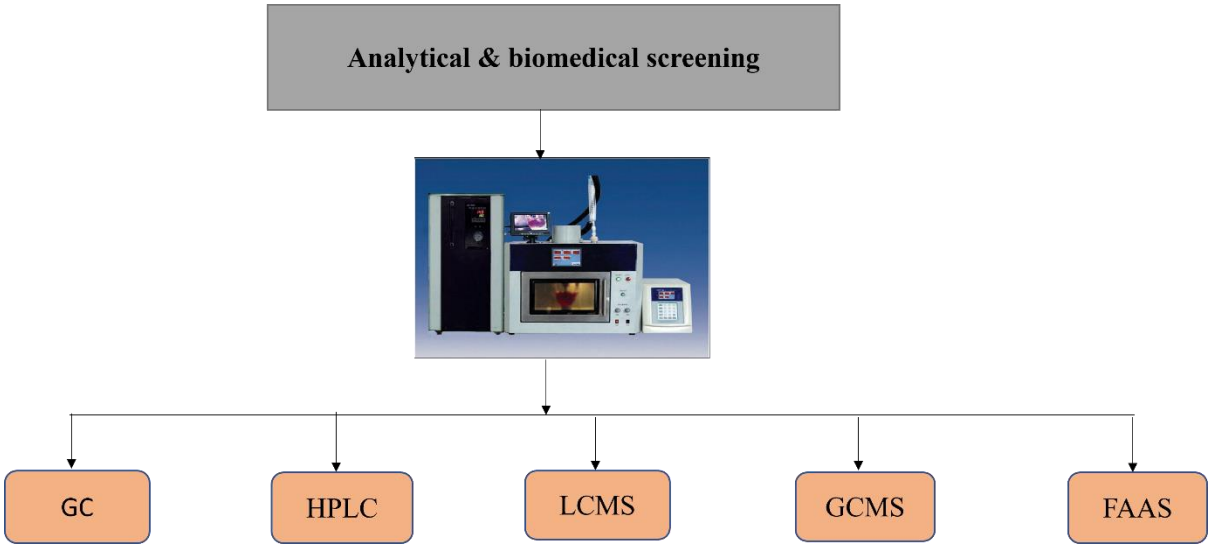


Fig. 2 Analytical & biomedical screening

3.1.2. High-Performance liquid chromatography (HPLC)

Compounds are commonly separated and quantified using High-Performance Liquid Chromatography (HPLC) based on their lipophilicity or polarity. However, heavy metals, which lack lipophilicity as ions, cannot be directly detected by HPLC. Instead, heavy metals can be analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC) after forming complexes with chelating agents during a pre-column derivatization process. [56].

Porphyrins can bind to a variety of metal ions, forming extremely stable 1:1 chelates that are difficult to break down under HPLC conditions. Using this property, a novel technique was developed to measure heavy metals in Chinese herbal remedies, combining reverse-phase high-performance liquid chromatography (RP-HPLC) with microwave digestion [57].

Tetra-(4-chlorophenyl)-porphyrin (T4-CPP) was subjected to microwave digestion and then mixed with lead, cadmium, mercury, nickel, copper, zinc, and tin ions from the samples to form chelates. After the chelates were extracted using a C18 cartridge and eluted with tetrahydrofuran (THF), they were separated using an RP18 column. This method effectively assessed the concentrations of lead, cadmium, mercury, nickel, copper, zinc, and tin in samples of Chinese herbal medicine, yielding promising results [58]. Additionally, a study shows that HPLC can efficiently quantify the concentrations of heavy metals found in metal-organic complexes produced during the manufacturing of an anis-type beverage. The waste generated contained various elements, including Fe (157.5 mg/L), Cu (82.5 mg/L), Zn (31 mg/L), and Ni (8.5 mg/L) [59]

3.1.3. Liquid Chromatography-Mass spectrometry (LC-MS):

Liquid Chromatography-Mass spectrometry (LC-MS) was used to analyse the pesticide residue in herbal plants. The parameters for sample processing and preparation were optimized in this study to select an appropriate extraction solvent from acetonitrile (ACN), ethyl acetate (EtAC), and their mixtures for residue analysis of several pesticides in various herbal plants, including dried mint, chamomile, and fennel seeds. By using a combination of ACN and EtAC, the number of pesticides detected with acceptable precision and accuracy in dried mint, chamomile, and fennel was 78, 288, and 298, respectively [60]. All samples underwent standard processing, which resulted in high particle sizes. These findings, along with the scanning electron microscope images obtained, demonstrated that dried mint is one of the most challenging herbal commodities for pesticide residue analysis. [61]

3.1.4. Gas chromatography-mass spectrometry (GC-MS):

Mass spectrometry is a highly efficient and sensitive technique used for molecular analysis. This method allows scientists to obtain detailed information about a molecule's molecular weight and structural characteristics. By ionizing chemical compounds and measuring their mass-to-charge ratios, mass spectrometry reveals crucial insights into the composition and behavior of various substances at the molecular level. This powerful tool is indispensable in fields such as chemistry, biochemistry, and proteomics, enabling researchers to identify unknown compounds and explore complex mixtures with remarkable precision. This method combines the advantages of mass spectrometry as a detection system with chromatography as a separation technique. In this process, compounds undergo ionization before being analyzed in the mass spectrometer. There are two ionization techniques commonly used with gas chromatography: Electron Impact (EI) and Electron Capture Ionization (ECI). ECI is typically employed to target negative ions (ECNI), while EI is primarily used for positive ions. EI produces consistent mass spectra with structural information that facilitates library searches, making it particularly useful for routine analyses. A GC-MS based study was conducted to evaluate the phytochemicals, biological activity, and heavy metal content of Reseda muricata and Marrubium vulgare cultivated in various regions of Saudi Arabia. Qualitative phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, phenols, and saponins in both R. muricata and M. vulgare. Additionally, gas chromatography-mass spectrometry (GC-MS) was used to analyze methanol extracts of these two plants to quantify the heavy metals [62]

3.1.5. Flame atomic absorption spectrometry (FAAS)

In atomic absorption spectrometry (AAS), light of a specific wavelength is directed at atoms in their ground state. These atoms can absorb the light energy, which causes them to transition into an excited state. The amount of light energy absorbed is proportional to the concentration of atoms present in the sample. To establish this relationship, a reference solution with known atom concentrations is typically used, often through basic regression analysis.

A typical Atomic Absorption Spectroscopy (AAS) setup consists of several key components: an atom source, a main light source, a monochromator, a detector, and an electrical system for data analysis. The light source can be either a hollow cathode lamp or an electrodeless discharge lamp, both of which are capable of producing reliable results. Typically, several lamps with different wavelengths are used to accurately identify various elements. Additionally, a multielement lamp can be employed to measure the concentration of multiple elements simultaneously without needing to turn off the lamp [63].

Flow Injection Mercury Systems (FIMS), which are atomic absorption spectrometers, are specifically designed to measure mercury levels, a common source of contamination. To minimize interference and enhance accuracy, FIMS utilize a high-performance single-beam optical system equipped with a solar-blind detector and a low-pressure mercury lamp. Before analyzing the sample, the atom source must generate free analyte atoms. One method to generate free atoms is by using the heat from an air-acetylene or nitrous oxide-acetylene flame. The sample is introduced into the flame at the burner head using a nebulizer within a spray chamber, which allows for the necessary atomization for analysis. The light beam passes through the flame, where it is absorbed based on the concentration of atoms. The contents of copper and manganese in herbal remedies have been analyzed using flame atomic absorption spectrometry [64].

4. Biomedical Screening of contaminants:

Chemical analysis presents several challenges. Physiological conditions, harvesting time, and storage conditions can all affect the levels of active components in a sample. Additionally, accurate analysis often requires large sample sizes. Furthermore, some analytical instruments, such as high-performance liquid chromatography (HPLC), capillary electrophoresis, and mass spectrometry, can be quite costly and may not be accessible in many laboratories. Chromosome counting and karyotyping are traditional cytogenetic techniques that can be utilized to differentiate medicinal materials and assess plant hybridity [65]. However, one disadvantage of these methods is that protein patterns can vary due to temporal and spatial gene expression, depending on the tissue type, developmental stage, and environmental conditions. Additionally, it can be challenging to identify distinct markers in closely related species, and the proteins in herbs are often prone to degradation after extended storage.

Individual genetic makeup is unique and is less influenced by factors such as age, physiological state, and environmental influences, making DNA molecules reliable indicators of relevant polymorphisms. One advantage of DNA markers is that a small sample size is sufficient for analysis, and the detection process is not restricted by the physical form of the sample [66]. DNA can be extracted from various parts of herbal materials, including leaves, stems, or roots. As a result, DNA fingerprinting can be very useful for evaluating and validating the species of the plant materials in question. Additionally, these methods can be employed to investigate various herbal therapeutic ingredients using their crude extracts or bioactive fraction-guided approaches.

5. Regulatory Considerations:

End customers can trust the safety, effectiveness, and quality of products due to the strict control of contaminants in raw medicinal herbs. Depending on national laws and classifications established by various regulatory bodies, medicinal plants may be categorized as either food (dietary supplements) or medical products. The classification of herbal remedies and medicinal plants can vary both within and between countries. In the United States, herbal remedies are generally less strictly regulated because they are classified as dietary supplements. In contrast, countries like China, Singapore, Canada, Australia, and the UK classify medicinal plants as medical products, which are subject to stricter regulations similar to those governing traditional medicines. The primary challenge to international trade in herbal medications and medicinal plants is the lack of uniform regulations, legal frameworks, and oversight agencies to effectively enforce these laws.

5.1 Heavy metals:

The World Health Organization (WHO) in 2007, along with the US Pharmacopoeia (USP) and the European Pharmacopoeia (Ph. Eur.), established several international regulations regarding the safety of medicinal plants. Additionally, several countries have implemented their own national regulations regarding the permissible levels of heavy metals in these facilities. This includes Canada, China, Malaysia, Singapore, Thailand, and India. Table 6 compares the allowable levels of heavy metals based on information provided by each country's national health authority. Generally, it is recommended to assess the heavy metal content of herbal materials both qualitatively and quantitatively, ideally using samples from multiple batches collected over several years. When determining permissible limits for potential metal intake, we consider the quantities of hazardous metals present in the product, the recommended or expected dose, and the results of relevant limit tests. To assess potential exposure within a toxicological framework, we compare it to the Provisional Tolerable Weekly Intake (PTWI) values for these hazardous metals. These PTWI values serve as reference limits set by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). [69]

Table: Limits of heavy metals in herbals

S.N	Heavy Metal	Permissible limits as per regulations (PPM)			
		WHO	USFDA	China Pharmacopoeia	Department of Ayush, India
1.	Cadmium	0.3	0.3	0.3	0.3
2.	Lead	10	10	5.0	1.0
3.	Arsenic	10	10	2.0	10
4.	Mercury	1.0	1.0	0.2	1.0
5.	Zinc	50	50	---	---

5.2 Pesticide residue:

There are currently no restrictions or guidelines for Maximum Residue Levels (MRLs) in medicinal plants. The Codex Alimentarius Commission (CAC) has released a global list of approved pesticides and their Maximum Residue Limits (MRLs) for spices. Many countries have developed national standards for residual limits in medicinal herbs, primarily based on the restrictions set for food commodities. For example, the Chinese National Pharmacopoeia Committee has recommended that the upper limit for banned organochlorine pesticides in Panax and American ginseng should be between 0.05 and 0.2 parts per million (ppm). Table 8 provides examples of national limits established for various organochlorine pesticides. The Acceptable Daily Intake (ADI) of a pesticide, along with the dietary factor associated with the consumed commodity, determines the Maximum Permissible Intake (MPI) for humans. Good Agricultural Practices (GAP)-compliant controlled field experiments provide the scientific basis for establishing Maximum Residue Limits (MRLs) for food products. According to the World Health Organization (WHO) guidelines from 1999, the toxicological evaluation of pesticide residues in herbal products should be based on a maximum of 1% of total intake from all sources, including food and drinking water. Consequently, further research is necessary to develop scientific guidelines for setting limits on herbal medicines. In the absence of specific MRLs for these products, the MRLs for medicinal herbs can be derived from those established for botanically similar plants. Currently, there are no globally acceptable maximum limits for PAHs and fumigants in therapeutic plants. Additionally, Europe has banned the use of ethylene oxide as a fumigant in herbal medications [73]

Table: Permissible limits of some organochlorine pesticides in medicinal herbs

S.N	Pesticide	Limits (mg/kg)				
		EP	USP & FA	Japan	China CP	GSMPP
1	DDT & isomers	1.0	1.0	0.2	0.2	0.1
2	Pentachloronitrobenzene PCNB	-	-	-	0.1	0.1
3	Aldrin and dieldrin (sum of)	0.05	0.05	-	0.05 (Aldrin)	0.02 (Dieldrin)
4	Hexacholrocyclohexane isomers	0.6	0.3	0.2	0.1	0.2
5	Heptachlor	0.05	-	-	0.05	-
6	Chordane	0.05	0.05	-	0.1	-

EP: European Pharmacopoeia,11th edition July 2022; USP: United state Pharmacopoeia May 2024; FA: Pharmacopoeia Argentina Vol 1; CP: Chinese Pharmacopoeia 2023; GSMPP: Green standard for medicinal plant and preparation included in Chinese Pharmacopoeia

5.3. Mycotoxins

European legislation has established legal limits for mycotoxins, specifically aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA), and fumonisins (FBs) in various food products. The only regulatory restrictions on aflatoxin (AF) and ochratoxin A (OTA) contamination in spices and herbs are specific in nature. According to EU standards issued in 2012, the maximum permissible levels of OTA are set at 15 mg/kg for spices, 20 mg/kg for Glycyrrhiza spp. (liquorice), and 80 mg/kg for liquorice extracts. Additionally, maximum levels for aflatoxins were established in 2011, allowing 2 mg/kg for aflatoxin B1 (AFB1) and 4 mg/kg for total aflatoxins in herbs. Other food items that are consumed directly are also subject to these restrictions, as noted in Table 7. It has been suggested to consider increased upper limits for herbs, proposing 5 mg/kg for AFB1 and 10 mg/kg for total aflatoxins, given that medicinal herbs are used significantly less frequently than food items. [75].

6. Summary & Conclusion:

Despite the long history and widespread use of medicinal plants as food and medicine, there has been limited research on the impurities present in these herbs. Most of the traditionally utilized medicinal and aromatic plants are native to countries such as China, India, and Southeast Asia. However, a significant barrier to the export of these herbs is the lack of national and international quality standards and protocols for monitoring both raw materials and finished products. Although the European Union and several other countries, including China, Japan, and India, have collaborated with FAO/WHO to create national and region-specific guidelines for Good Agricultural and Collection Practices (GACP) for medicinal plants, these regulations are often not adhered to in developing nations. To ensure the proper collection of plant materials and their cultivation in environments free from or within acceptable limits of toxic heavy metals, mycotoxins, pesticide residues, and other hazardous substances, it is crucial to implement and closely monitor these guidelines.

The establishment and implementation of an international monitoring organization for quality assurance, along with a surveillance program for medicinal herbs, is essential due to the numerous reports of toxins found in these plants. When such a plan is enacted globally, it will become easier to identify therapeutic herbs that contain clinically significant toxins and to trace the individual farms or factories that are responsible for the contamination. Additionally, whether samples are sourced from cultivated plants or wild ones, they should always be tested for impurities. To better understand the patterns of contamination in medicinal plants linked to industrial and agricultural pollution, further research is necessary.

Research on the monitoring and regulation of harmful heavy metals, such as lead (Pb), cadmium (Cd), arsenic (As), and chromium (Cr), as well as mycotoxins, persistent pesticides—particularly organochlorines and organophosphates—and polycyclic aromatic hydrocarbons (PAHs) in unprocessed medicinal plants is crucial. Achieving this goal requires collaboration among all stakeholders in the industry to establish national and international regulations that define acceptable upper and lower limits for contaminants and residues in medicinal plants. Such measures would enhance global trade and protect consumer health.

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