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Review Article

Detection of Adulteration in Herbal and Nutraceutical Products: Analytical Approaches, Regulatory Perspectives, and Emerging Technologies

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Besides having a positive effect on consumer health, herbal medicines combined with nutraceutical products make a major contribution to the global healthcare market where both types of remedies are very popular due to their presumed harmless nature, cultural acceptance, and easy availability. Unfortunately, these increasing demands have resulted in the parallel spread of product adulteration, like the use of cheaper substitute fillers to lower costs of the product, substitution of a species with another, and in some cases the very stealthy use of synthetic pharmaceutical agents or heavy metal contamination to the products. The use of such adulterated products puts the consumers' health at risk, makes it hard for them to benefit from the medicinal product and may ultimately, lead consumers to lose belief in herbal and other traditional types of medicine. This review compiles the information we have at present on the issue of the nature and prevalence of the adulteration of herbal and related products and discusses critically the detection methods including both macro- and microscopic evaluation of pharmacognosy, chromatographic fingerprinting (TLC HPTLC HPLC, GC-Max), spectroscopic techniques (UV-Vis, FTIR. NIR Raman, NMR), mass spectrometry platforms, DNA barcoding and metabarcoding techniques for heavy metals, elemental analysis, and new developments in biosensor-aided and artificial intelligence-based methods. And, the article offers an overview of the regulatory setup covering World Health Organization (WHO) recommendations, Indian AYUSH (Ayurveda, Yoga & Naturopathy Unani Siddha and Homeopathy) national legislation, and pharmacopoeials. It is pointed out in the review that single methods are inadequate for the purposes of quality control, that means a combined multi-technique strategy which also benefits from uniform worldwide laws and continuing technological advancements seems to be the most effective way of guaranteeing safety and authenticity of herbal and dietary product categories.

Keywords: Herbal Medicine Adulteration, Nutraceutical Quality Control, DNA Barcoding, Chromatographic Fingerprinting, Spectroscopy, Heavy Metal Contamination, Regulatory Standards

INTRODUCTION

Herbal medicines and nutraceuticals have been used by human societies for thousands of years and continue to constitute a significant proportion of health care consumed by large segments of world populations today most of all in the developing world. They have also been extremely popular in the industrial world due to consumer perception that natural is better than synthetic. Really many herbal products are derived from intricate and variable plant sources in complex and lengthy supply chains, and that they are less stringently regulated than modern pharmaceutical drugs, combine to create an environment that allows adulteration of herbal products to thrive [1]. Adulteration is any procedure by which an authentic material has been replaced, mixed, or diluted with a substance that is not declared on the label. Forms of adulteration can include botanical substitution with morphologically similar, pharmacologically inactive species or artificially active adulterants, addition of drugs to boost perceived efficacy, microbial, or heavy metal

contamination, or simple economic adulteration with cheap fillers [2].

Adulteration isn't just a financial scam adulterated herbal and nutraceutical products have been related to events as severe as stimulants concealing cardiotoxic substances, as well as drug-induced liver and kidney damage, and heavy-metal intoxication [3]. The problem is widespread: molecular authentication studies carried out in multiple countries have estimated that up to a third of commercial herbal products aren't true to their labelled botanical source [4]. The absence of pre-market efficacy and safety testing for herbal and nutraceutical products, versus conventional drugs, means that precise, scientifically valid analytical detection of adulteration is required in this field to provide the facts for safe use of these products, and to maintain the integrity of traditional medicine systems [5].

This review intends to give a comprehensive technical overview (i) of the major types and drivers of adulteration of herbal and nutraceutical products, (ii) of the current regulatory standards tied to quality and (iii) of the major methods, ranging from classical pharmacognostic to modern molecular and artificial intelligence-based approaches, currently employed to screen for adulteration, with representative case studies used throughout to highlight both the strengths and limitations of each.

2. Types and Sources Of Adulteration

2.1 Species Substitution and Botanical Admixture

The substitution of an authentic medicinal species with a morphologically or taxonomically related, but pharmacologically distinct, species is among the most extensively documented forms of adulteration. This may occur unintentionally, owing to confusion of vernacular names or the use of "controversial drugs" with overlapping common names in traditional systems, or intentionally, for economic gain when the authentic material is scarce, endangered, or costly to procure [6]. Large-scale DNA-based surveys of the herbal trade have found that adulteration rates vary much by product category and country of origin, with some regions and product types mainly multi-ingredient polyherbal formulations showing markedly higher rates of substitution than single-ingredient raw materials [7]..

2.2 Chemical and Pharmaceutical Adulteration

A direct risk to consumer safety is posed by the hidden addition of synthetic active pharmaceutical ingredients (APIs) to herbal and nutraceutical products, intended to create a rapid and noticeable pharmacological effect that strengthens consumer confidence in the product's effectiveness. This practice is most often observed in three types of products: weight-loss supplements, sexual-enhancement products, and body-building or sports-performance supplements [8]. In weight-loss supplements, undeclared substances such as sibutramine (and related analogues) phenolphthalein, fluoxetine, fenproporex, and amfepramone have been repeatedly found in various national markets [9]. For sexual-enhancement products, phosphodiesterase-5 (PDE-5) inhibitors including sildenafil vardenafil tadalafil, or their analogues are the most frequently detected undeclared APIs [10].

2.3 Heavy Metal and Microbial Contamination

Herbal raw materials might contain metals such as lead, cadmium, mercury, arsenic, nickel, and chromium if present in contaminated soil, water, or processing equipment, or such metals can intentionally be added to a product during processing as part of certain traditional practices [11]. In samples of adaptogenic herbal preparations containing Withania somnifera, Rhodiola rosea, Panax ginseng and Schisandra chinensis, most were found to contain lead and nickel above acceptable levels, with lead in processed tablets exceeding legally permissible levels by as much as 235% [12]. Market testing of dietary supplements marketed in the United Arab Emirates revealed several contaminants in a significant proportion of the samples, with several samples exceeding legal limits for arsenic, lead, and mercury by a wide margin [13].

2.4 Economic (Filler and Excipient) Adulteration

While less inherently dangerous, but high-volume adulteration is by dilution of authentic botanical material with inert extenders, starches, or lower-value botanical material to cut production costs while retaining the visual characteristics of the marketed product. Due to the absence of discernible safety signals, this type of adulteration is often identified via fingerprinting-based chemical analysis or spectroscopic screening and not by adverse event reports [14].

3. Regulatory Framework For Herbal And Nutraceutical Quality

Herbal medicines are locally regulated, not globally, consistent with the legal and cultural standing of traditional medicine system in each location. Guidelines on quality control from the field to the patient have been issued by the World Health Organization (WHO) in the form of Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) regulations, which are not mandatory for countries to enforce [15].

In India, herbal medicines created in the Ayurveda, Siddha, and Unani systems are regulated under the Drugs and Cosmetics Act, 1940 and Rules, with manufacturing standards in Schedule T Good Manufacturing Practice rules supervised by the Ministry of AYUSH [16]. The Pharmacopoeial Commission for Indian Medicine and Homoeopathy published pharmacopoeial standards for hundreds of single drugs and compound formulations in the Ayurvedic, Siddha, and Unani systems, and a 2022 agreement between the Pharmacopoeial Commission and the Indian Pharmacopoeia Commission under the "One Herb, One Standard" initiative aims to establish common quality standards for herbal medicines nationally [17]. In India, a voluntary AYUSH quality mark certification program, operated by the Quality Council of India since 2009, is another avenue through which manufacturers that meet either India's national GMP standards or WHO standards are distinguished, although not all manufacturers use the scheme [18].

In the USA, Dietary Supplements and most herbal and nutraceutical products are dealt with by the Dietary Supplement Health and Education Act (DSHEA) scheme, which takes proof of safety before market entry to be mostly provided by the manufacturers, rather than being pre-approved by the FDA. Instead, the authority of the FDA is limited to the post-market removal of identified unsafe or adulterated products [19]. This 'reaction rather than action' regulatory asymmetry has been recognised as a structural factor driving the continued presence of adulterated products in the US supplement industry [20].

4. Analytical Methods For Detection Of Adulteration

4.1 Macroscopic and Microscopic (Pharmacognostic) Evaluation

Classical pharmacognostic evaluation which includes macroscopic assessment of organoleptic properties and microscopic analysis of diagnostic anatomical features like trichome structure, stomatal configuration, and vascular patterns remains the primary, most cost-effective approach for authenticating raw botanical materials. Yet, its reliability decreases Really once materials are powdered, extracted, or otherwise processed, as diagnostic anatomical features are often lost during these procedures [21]. Systematic reviews of commercially available herbal products authenticated by microscopy have found overall authenticity rates below two-thirds, with the remainder showing signs of adulteration. This highlights both the ongoing value and the practical limitations of morphology-based identification for finished products [22]..

4.2 Chromatographic Fingerprinting Techniques

Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) are still the mainstay for rapid, inexpensive qualitative screening of herbal extracts, mainly when multiple samples and reference standards are loaded on a single plate [23]. HPTLC fingerprinting has been used to generate pharmacopeial quality standards for many single- and polyherbal Siddha and Ayurvedic formulations [24] and combined with the chromatographic fingerprint pattern with pharmacognostic and physicochemical parameter data to produce composite authentication profiles. High-performance liquid chromatography (HPLC) usually coupled with diode-array or mass spectrometric detection is more highly resolved and sensitive and remains the workhorse for quantification of marker compounds or detection of undeclared synthetic adulterants in complex herbal

matrices [25]. Gas chromatography with MS detection (GC-MS) works in complementary fashion for volatile or semi-volatile adulterants, including some synthetic stimulant adulterants and residual solvents [26].

4.3 Spectroscopic Methods

UV-Vis FTIR NIR, and Raman spectroscopies have become reliable rapid, non-destructive screening methods with minimal sample preparation. FTIR and Raman techniques are mainly useful for qualitative screening of suspected PDE-5 adulterated herbal food supplements, allowing the screening to be performed in a rapid, low-cost fashion as an alternative to chromatographic confirmation, while maintaining enough specificity to indicate, which samples need to be validated with GC-MS [27]. On top of NIR spectroscopic methods that can discriminate authentic herbal powders from common adulterants across a broad spectrum of species using pattern recognition methods like principal component analysis and partial least squares regression increasingly machine learning classifiers have been used [28]. For instance, an NIR-based approach using optimized spectral preprocessing plus a hybrid kernel extreme learning machine improved classification of genuine American ginseng (Panax quinquefolius) powder adulterated with Angelica dahurica, Astragalus membranaceus, Panax notoginseng and wheat flour to >95% [29]. The use of FT-NIR spectroscopy with chemometric modelling has like that shown potential to detect adulteration of ginger powder, something simple chromaticity (colour) measurements alone could not reliably achieve [30]. NMR spectroscopy, while more labour- and cost-intensive beyond requiring specialized instrumentation, brings the ability to simultaneously detect, identify, and quantify multiple adulterants during a single spectral acquisition, which could prove useful in untargeted screening for weight-loss and other high-risk supplement categories [31].

4.4 Mass Spectrometry-Based Platforms

Hyphenated mass spectrometry-based approaches (liquid chromatography-mass spectrometry (LC-MS), ultra-performance liquid chromatography-QTOF-MS (UPLC-QTOF-MS), capillary electrophoresis-mass spectrometry, etc.) offer the sensitivity and structural resolution necessary for the detection of low-level, structurally altered ("designer") synthetic adulterants potentially missed by crude chromatography or spectroscopy-based screens [32]. They are mostly necessary for analysis of analogue compounds intentionally designed by adulterators to get around the applicability of routine reference-standard compound based screens (a reproducible occurrence well described in the dietary supplement literature) [33].

4.5 DNA-Based Molecular Authentication

Metabarcoding has been identified as a valuable technique for herbal product testing because the classification target short, standardised segments of DNA (such as rbcL, matK, ITS2, and psbA-trnH in plants) allows species-level identification to be achieved without regard to the physical or chemical condition of the material, the external environment or the date or age of the tissue [34]. When combined with polymerase chain reaction (PCR) amplification, and increasingly next-generation sequencing-based metabarcoding, such DNA-based methods can identify adulteration even in finished multi-ingredient polyherbal formulations in which morphological and some chemical identification markers are unusable [35]. Commercial Brahmi products, a key nervine tonic used as a formulation of Bacopa monnieri or Centella asiatica, were examined through a DNA metabarcoding coupled with HPLC analysis and found to have marked differences between the label and the DNA-detected plant constituents for the samples tested [36]. In a related study that was only targeting a specific product, researchers used DNA metabarcoding to identify the adulterants in the traditional Chinese multi-component formulation Renshen Jianpi Wan, and this method was able to distinguish between genuine Panax ginseng and the more affordable adulterant, Panax quinquefolius [37]. In reviews on the Indian herbal products market, DNA barcoding combined with chemical analysis of marker compounds has been found to be a much more reliable means of identification than either approach alone [38].

4.6 Elemental Analysis for Heavy Metal Contamination

The main methods employed to determine trace and heavy metal contamination in herbal and nutraceutical raw materials and finished products are flame and graphite-furnace atomic absorption spectrometry (FAAS/GFAAS), inductively coupled plasma mass

spectrometry (ICP-MS), and dedicated mercury analysers [39]. Since the permissible limits of elements like lead, cadmium, arsenic, and mercury differ between pharmacopoeia and national regulations, elemental analysis results are generally expressed compared to the prescribed limit of the country of sale of the product. This approach contributes to the disparity in world-wide enforcement [40].

4.7 Emerging Technologies: Artificial Intelligence, Chemometrics, and Biosensors

These days, the integration of artificial intelligence (AI) and machine learning algorithms with spectroscopic and image data has become one of the fastest growing disciplines of adulteration detection research. Deep learning algorithms applied to spectral or digital image data can extract discriminant features automatically without a need for a large amount of manual feature engineering, often provide classification accuracy superior to conventional chemometric methods, like linear discriminant analysis [41]. An AI aided method of digital image recognition coupled with near-infrared spectroscopy for the detection of Bajiaohuixiang (Illicium verum) herbal powder could discriminate adulterated samples effectively in binary and multiple classes of adulteration and also predict accurately in external validation performed with both prepared and real market samples [42]. More holistic overviews of food and plant-based fraud detection technologies point to the increasing importance of artificial intelligence (AI) predictive analytics, in combination with blockchain and IoT-enabled traceability systems, as a means for offering integrated end-to-end supply chain transparency as an alternative to analytical detection at point-of-testing [43]. Development is also underway in sophisticated biosensor platforms that add electrochemical or optical sensing with machine learningbased signal processing to result in low-cost, field deployable screening tools that may be used to quickly identify likely suspect samples for more definitive, laboratory-based testing [44]. Summaries of technology development for food and plant-based fraud like that suggest the next generation of biosensors, immunoassays, and portable instrumentation, combined with AI, are on the cusp of offering real-time, on-site detection possibilities that cannot be achieved by traditional laboratory-based assays [45].

5. CASE ILLUSTRATIONS

5.1 Weight-Loss and Sexual-Enhancement Supplements

The Tainted Dietary Supplement Database of the United States Food and Drug Administration identified 1,068 unique adulterated products with active pharmaceuticals across a 14-year monitoring period, with PDE-5 inhibitors predominating among sexual-enhancement products and sibutramine and its analogues among weight-loss products [46]. A regional survey of weight-loss supplements available in the United Arab Emirates showed that a significant percentage of the tested formulations contained sibutramine, phenolphthalein, or fluoxetine [47]. Interestingly, longitudinal monitoring has indicated that products previously identified and reformulated by industry contained a different undeclared adulterant [48].

5.2 Adaptogenic and Traditional Botanicals

Adaptogenic herbal supplements, commonly used for their stress modulating capabilities, had a mostly high percentage of heavy metal contamination (although the levels of nickel in the Indian sourced adaptogen supplements tested were quite a bit higher than samples from China when tested comparatively [49]). This demonstrates that the risk of contamination, is not only due to manufacturing practices, but also determined by the geochemical parameters of the locality in which the plants are cultivated, further reinforcing the need for raw-material traceability systems.

5.3 Polyherbal Traditional Formulations

For more complex multi-herbal traditional combinations, where the discrete constituent herbs are normally not identifiable once the formulation has been made up, authenticating the formulation is more difficult. DNA metabarcoding of formulations like Renshen Jianpi Wan and Brahmi-labelled products showed that molecular techniques could identify the identity of species in the complex multiple-herb mixture whereas this would not normally have been possible with only analysis of the chromatogram to determine marker components [50].

6. CHALLENGES AND LIMITATIONS

Despite many advances in technology, several fundamental issues continue to hinder the practical implementation of adulteration detection. First, all currently available analytical methods and approaches, from the technological perspective, have their shortcomings: chromatographic and spectroscopic methods can detect chemical and pharmaceutical adulterants but not species substitution without reference libraries, while DNA methods can provide species ID but cannot quantify undeclared synthetic drugs [51]. Second, as adulterers become increasingly sophisticated, they will look to circumvent such reference-based screening approaches using several strategies, such as engineered analogue compounds and matrix-matching approaches, which will demand an ongoing process of re-optimising the reference libraries [52]. Third, the costs and requirements of instrumentation for high-end analytical techniques, such as UPLC-QTOF-MS, NMR or next-generation sequencing (NGS) based metabarcoding, may be out of reach of many small and medium-sized manufacturing firms, and may require considerable technical expertise in resource-poor laboratories [53]. Fourth, in the absence of regulatory coordination and the voluntary adoption of quality certification schemes, validation of analytical approaches is difficult and few tools are yet consistently used by producers or regulators [54].

7. FUTURE DIRECTIONS

Several converging trends indicate promising avenues for improving adulteration detection in the coming decade. Multi-omics and hyphenated methods that integrate DNA metabarcoding with chromatographic marker quantification are increasingly seen as providing more comprehensive authentication than either genomic or chemical techniques alone, as they address both species identification and the presence of undeclared chemical substances [55]. The ongoing development of portable, field-ready spectroscopic and biosensor tools, improved by cloud-based AI classification systems, raises the possibility of real-time screening during raw material procurement, rather than limiting testing to centralized laboratories [56]. The use of blockchain-enabled supply chain traceability, combined with periodic analytical verification, has also been suggested as a supplementary approach to reduce opportunities for adulteration to enter the supply chain, rather than depending only on detection at later stages [57]. Also, harmonizing pharmacopoeial standards and allowable contaminant limits across major regulatory regions, building on efforts like India's "One Herb, One Standard" initiative, could address current inconsistencies in enforcement that allow adulterated products to remain in markets with less stringent oversight [58].

CONCLUSION

Adulteration of herbal and nutraceutical products continues to pose a complex and ongoing risk to consumer safety and the reputation of traditional and complementary medicine systems globally. The evidence presented indicates that adulteration occurs across a broad range, from lower-risk economic substitution with inactive fillers to higher-risk contamination involving undeclared synthetic pharmaceuticals and toxic heavy metals. No single analytical method can fully address all these risks; instead, robust quality assurance relies on the combined use of pharmacognostic chromatographic spectroscopic, mass spectrometric, molecular, and elemental analytical techniques, with artificial intelligence increasingly aiding data interpretation. Strengthening and harmonizing regulatory systems internationally is also essential, ensuring that analytical progress is matched by consistent enforcement. Ongoing investment in accessible, portable detection tools, with coordinated global regulatory measures, offers the most promising approach to ensuring that herbal and nutraceutical products consistently provide the safety and efficacy expected by consumers.

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