

# A Discussion of the Recent Developments Made Towards the Standardization of Herbal Medicines

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**Abstract:** Herbal remedies are now widely accepted as effective treatment options for a number of illnesses. One of the biggest challenges facing scientists is the creation of reliable, genuine analytical techniques that can profile the phytochemical content. These techniques include quantitative studies of marker/bioactive chemicals and other important elements. Establishing a standardized biological activity, chemical profile, or even just a quality assurance procedure for the manufacture and manufacturing of herbal medications requires standardization. It is crucial to follow WHO-specific protocols for evaluating the quality, safety, and effectiveness of herbal medicines before attempting worldwide harmonization. An overview of the many methods used for standardizing herbal nanomedicines and for extracting and characterizing herbal medications is presented. Moreover, reports have been made regarding the enhanced bioavailability of phytosomes, the potential of metabolomics in the creation of better phytotherapeutic agents, the use of bhasma as a metal nanocarrier drug delivery system, the use of DNA-based molecular markers to identify adulterants, and the use of SCAR markers to authenticate and separate herbs from their adulterants. In regard to herbal medications, it has been discussed how high-value herbal compounds can be extracted using microwave-assisted extraction and supercritical phase extraction technology, followed by standardization using a variety of spectroscopic, chromatographic, and thermogravimetric techniques used singly or in combination. It is also stated that polarographic and capillary electrophoresis methods have contributed to the standardization of herbal medications. Chinese herbal medicines with a nanotechnology foundation have higher solubility and bioavailability.

**Keywords:** Herbal Drug Standardization, Standardization Advances, Quality Control, Traditional Medicine

## I. INTRODUCTION

Medical, nutraceutical, and cosmetic products made from plants are becoming more popular. They are available over-the-counter at health food shops and pharmacies as self-medication or as non-allopathic prescriptions<sup>1, 2</sup>. Herbal medications, which are used in both industrialized and developing countries' healthcare systems, are complex chemical combinations produced from plants that perform poorly when taken orally. The WHO estimates that 80% of people worldwide use herbs and other traditional medicines for basic medical needs. Herbal remedies for liver diseases, diabetes, arthritics, coughs, memory enhancers, and adoptogens are becoming common. Medical herbal products, processed plant material, and raw plant material are classified by the WHO. Herbal treatments include raw plant material, plant preparations, aerial or subterranean plant parts, or other plant material in combination. As people globally return to natural cures, herbal drugs have grown in popularity<sup>6</sup>. People use tablets, capsules, powders, teas, and extracts derived from fresh or dried plants to improve their health. Many people use herbal treatments without a prescription, believing them safe. However, some may interact with other drugs, fail, or cause health problems. Industry and organizations dealing with ayurveda and herbal products must check herbal preparation quality. Standardization of herbal formulations is essential for assessing pharmaceutical quality based on active ingredient concentration. The public's growing use of botanicals (drugs and other plant-based products) is spurring initiatives to assess their health benefits and set manufacturing and quality standards. Herbal business rules are necessary and rigorous. India's herbal pharmaceutical regulations and those of the US, China, Australia, Brazil, Canada, and Germany have been recorded. Before a herbal product is sold, WHO standards require safety testing.

### **Herbal drug technology**

The process of turning botanical materials into medicines via standardization, quality control, and the appropriate blending of contemporary scientific methods with traditional wisdom is known as herbal drug technology. A variety of drug delivery systems for herbal pharmaceuticals have also been documented. The content and qualities of conventional pharmaceutical goods and herbal medical products might differ, and a growing number of regulatory authorities are focusing on standardizing herbal formulations due to adverse reaction reports. In this regard, accurate identification and quality control are crucial preconditions for guaranteeing the consistent quality of herbal medication, which enhances its safety and effectiveness<sup>12</sup>. The several methods used in the extraction, characterisation, and standardization of herbal, polyherbal, and nanoherbal medications are covered in this review article.

### **Herbal drug standardization**

Standardization ensures a consistent amount, quality, and therapeutic effect of components in every dose. A herbal product is not scientifically valid unless it has been validated and detailed to ensure repeatability. Furthermore, direct toxic effects, allergic reactions, pollutant effects, and herbal drug combinations have been reported<sup>6</sup>, which may be lethal. The phytochemicals in a plant influence its medicinal effectiveness. Developing trustworthy, authentic phytochemical analysis methods is a major task for scientists. Quantitative investigations of bioactive compounds and indicators are used. Standardization is a first step in establishing a standardized chemical profile, biological activity, or quality assurance procedure for herbal medication manufacturing<sup>13</sup>. Authenticating herbal medications and identifying adulterants from real medicinal plants is crucial for public health, pharmaceutical companies, and reproducible natural medicine.

### **Conventional methods for standardization of herbal formulation**

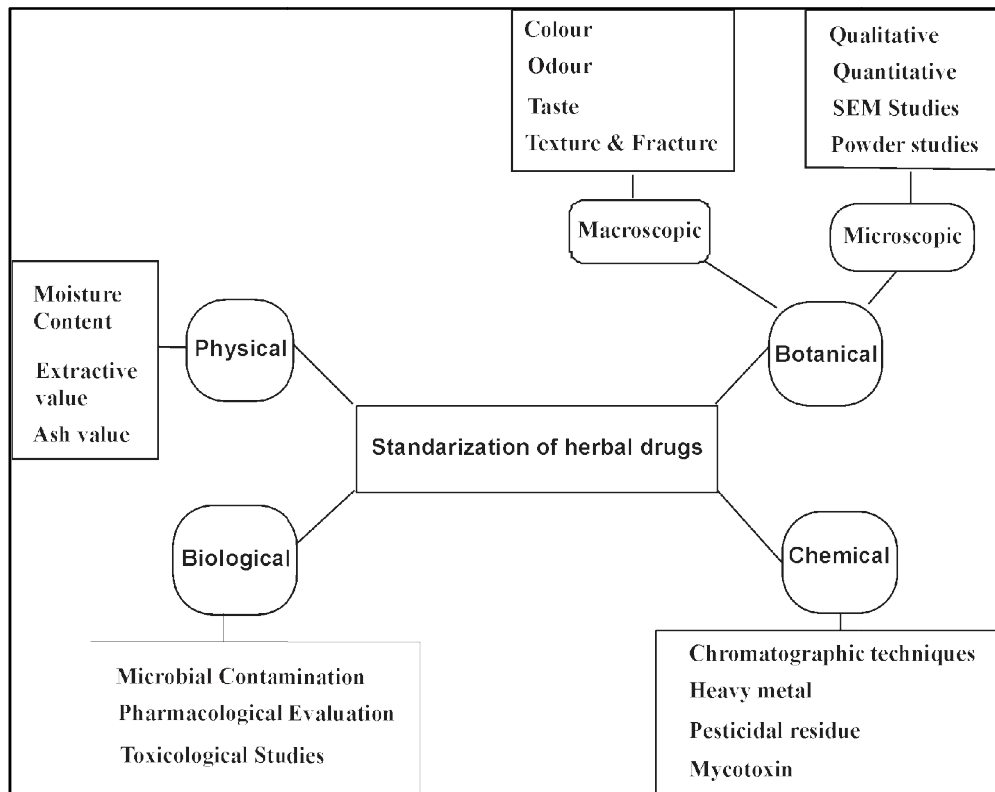
Herbal raw medicine standardization encompasses entire plant biological activity, botanical authentication, microscopic and molecular analysis, chemical composition identification by several chromatographic techniques, and passport data of raw plant pharmaceuticals. Many researchers have examined the macroscopic, microscopic, and chemical profiles of herbal materials for quality control and standardization. Medicinal plant materials are identified macroscopically by shape, size, color, texture, scent, and taste. Microscopy is based on comparative microscopic study of powdered herbal medications. Since light and scanning electron microscopes (SEM) are used to standardize herbal medications, microscope technology has enhanced its accuracy and capacity for detecting herbal medicine raw materials<sup>18</sup>. Herbal drugs are also standardized using cutting-edge methods including chromatography, spectrophotometry, electrophoresis, polarography, and molecular biomarkers in fingerprints.

### **Standardization of herbal formulation**

The use of Good Manufacturing Practices (GMP) is necessary for standardizing herbal formulation. Furthermore, it is thought to be crucial to research a number of factors, including pharmacodynamics, pharmacokinetics, dose, stability, self-life, toxicity assessment, and chemical profiling of the herbal formulations. Other equally significant issues include pesticide residue, aflatoxine concentration, heavy metal contamination, and the use of Good Agricultural Practices (GAP) in the standardization of herbal medicines.

### **Standardization of polyherbal formulations**

Standardization plays a crucial role in preserving and evaluating the quality and safety of polyherbal formulations, which are mixtures of many herbs to achieve the intended medicinal effect<sup>41</sup>. Based on physical attributes, physico-chemical qualities, and organoleptic features, the polyherbal formulation for hyperlipidemia has been standardized<sup>42</sup>. A US patent has been awarded for the formulation and standardization of a polyherbal formulation (Artrex®) that contains four botanicals and was created for the treatment of arthritis utilizing contemporary scientific instruments and recognized indicators.



**Figure : A schematic representation of herbal drug standardization**

There have been reports of the standardization of various marketed herbal and polyherbal formulations, such as Madhumehari Churna (Baidynath), which contains a mixture of eight herbal antidiabetic drugs: *Momordica charantia* (seeds), *Syzigium cumini* (seeds), *Trigonella foenum* (seeds), *Azadirachta indica* (leaves), *Embllica officinalis* (fruits), *Curcuma longa* (rhizomes), *Gymnema sylvestre* (leaves), and *Pterocarpus marsupium* (heart-wood). Dashamularishta is a traditional formulation that is used to normalize physiological processes after childbirth. Gokshuradi Churna, Megni, Jawarish-e-Darchini<sup>47–49</sup> have been documented. However, a lot of polyherbal formulations still need to be standardized since they are often utilized only for their ethanobotanical purposes<sup>50</sup>. Standardization reduces variance between batches and ensures the polyherbal formulations' acceptability, safety, effectiveness, and quality. Methiorep Premix, a blend of herbs including *Cicer arietinum*, *Phaseolus mungo*, *Mucuna pruriens*, *Triticum sativum*, and *allium cepa*, along with a richer source of protein with highly bioavailable methionine, has been suggested as a safe product to supplement basal diet on a regular basis and replace synthetic methionine in poultry rations. The identity, purity, and potency of the polyherbal formulation were determined using TLC and HPTLC fingerprint profiles, which were also used to establish criteria for this Ayurvedic preparation.

### DNA fingerprinting technique

Standardizing herbal medications using DNA analysis has been proven. This approach may distinguish between real phytochemically similar medications and tainted or substituted ones. Reports show that phytochemical composition depends by plant component, physiology, and environment, but the DNA fingerprint genome is the same. DNA fingerprinting may also identify adulterants in processed samples due to commercial herbal drugs' intact genomic DNA specificity. In recent years, various studies have examined the association between closely related species' phytochemical composition and DNA markers. *Glycerrhiza*, *Echinacea*, *Curcuma*, and *Arabidopsis* have shown interspecies variation using random amplified polymorphic and random fragment length polymorphism DNA markers. Molecular methodologies and analytical tools were employed to create a comprehensive botanical characterization system for industrial quality control. Since DNA markers may replace flawed proteins with normal

ones, they can identify cells, persons, and species. These signs help cure certain diseases and distinguish between genuine herbal treatments and modified pharmaceuticals.

### **ISSR (Inter-Simple Sequence Repeat)**

ISSR, a PCR-based DNA fingerprinting technology, may describe genetic fingerprinting, tag genes, detect clonal variation, conduct phylogenetic analysis, identify genomic instability, and assess hybridization. ISSR markers identify *Cannabis sativa* and *Arabidopsis thaliana* L. Heyne from their polluted species. Sequence-characterized amplified region (SCAR) markers may accurately and effectively identify herbal goods from adulterants. SCAR markers<sup>58</sup> may also differentiate similar-looking plant species. DNA-based genetic markers may distinguish *Taxus wallichiana*, *Azarchdichta indica*, *Juniperus communis* L., *Codonopsis pilosula*, *Allium schoenoprasum* L., and *Andrographis paniculata* accessions from different locales.

Herbal medication standardization guidelines WHO rules are followed as follows: a) botanical traits; b) physical and chemical identification; c) fingerprint chromatography; d) detail-toxicity pesticide residues; e) viable count total microbial contamination; and e) pathogens such *E. coli*, *Salmonella*, *P. aeruginosa*, *S. aureus*, and *Enterobacteriaceae*

Phytosomes/pharmacosomes: An new herbal medication delivery system Pharmacosomes, or phytosomes, are drug-phospholipid complexes with herb-derived active chemicals. Pills, capsules, powder, granules, aqueous microdispersion, lotion, gel, cream, solution, suspension, emulsion, syrup, lotion, gel, cream, or chewable tablets are possible. *Silybum Marianum*, *Ginkgo Biloba*, and ginseng outperform conventional herbal remedies. In human clinical studies, phytosomes had better absorption than flavonoids and polyphenols in herbal preparations. Many phytosomal herbal drug delivery techniques have been reported<sup>65</sup>. Researchers showed greater bioavailability in curcumin, silybin, flavan-3-ol catechins, and proanthocyanidin polyphenol phytosomes. This impact was caused by polyphenol molecules interacting with phosphatidylcholine<sup>66</sup> phospholipid molecules. The main uses of phytosomal herbal drug delivery systems are: supply systemic antioxidants (mainly flavonoids, terpenoid, and polyphenolic components); treat cancer, liver, blood pressure, and skin illnesses; and preserve the brain lining.

### **Standardization of herbal nanomedicines**

In order to achieve the intended therapeutic effect, active phytoconstituents may be included with the aid of herbal nanotechnology. Many well-known herbal extracts, such as milk thistle, ginkgo biloba, grape seed, green tea, hawthorn, and ginseng, have been shown to have increased solubility, stability, bioavailability, and pharmacological activity when administered using nano dosage forms, such as polymeric nanoparticles, liposomes, proliposomes, solid lipid nanoparticles, and nanoemulsions<sup>67,68</sup>. Additional benefits of herbal nanomedicine include enhanced tissue macrophage dispersion, protection against toxicity, prolonged administration, and defense against chemical and physical deterioration. In wistar rats, a 150g dosage of *Ocimum sanctum* extract-based silver nanoparticles exhibited the highest level of antibacterial activity. The incorporation of an electrospun antibacterial nanofibrous mat in the herbal medication offered a possible utility as a wound dressing. There have been reports of Chinese herbal medicine patents including nanotechnology, and the growth of these patents in China was caused by the delusion that biomedical technology was advancing rapidly.

### **Bhasma as a nanoherbal medicine technology**

Bhasmas are Ayurvedic metallic preparations that are taken with milk, butter, honey, or ghee to enhance the body's biocompatibility and reduce the negative effects of metals. These preparations use metal to act as a nanocarrier for drug delivery and are widely recommended for treating a range of chronic ailments. Twenty metallic-based bhasmas, including calcium, iron, zinc, mercury, silver, potassium, arsenic, copper, tin, and gemstones, were subjected to neutron activation analysis, which verified their purity. Elements like Na, K, Ca, Mg, V, Mn, Fe, Cu, and Zn were detected in microg/g quantities, while Au and Co were found in ultratrace (ng/g) amounts. The estimate and characterisation of bhasma have been done using a variety of methods, including energy dispersive spectroscopy, transmission electron microscopy, scanning electron microscopy, and atomic force microscopy.

### **Regulation of herbal medicines**

In order to assist member states in developing national policies on traditional medicine and researching its potential benefits, including effectiveness, safety, and assessment, WHO has developed recommendations. The Drugs and Cosmetics Act, 1940 governs traditional medicine in India, and state governments carry out the Act's requirements. India is the world's largest supplier of herbs, according to the first Indian National Health Policy of 1983, and medications should be standardized. In order to create pharmacopeial standards for Ayurvedic remedies, the Indian government's Department of AYUSH developed a central initiative to create standard operating procedures for the manufacturing process. Compared to India, the regulations governing herbal medicinal items are stricter in Europe and the US.

### **Pharmacovigilance of Herbal Medicines**

Drug side effects and other difficulties are identified, evaluated, understood, and prevented via pharmacovigilance. Chemical, herbal, conventional, complementary, biological, vaccines, blood products, and medical gadgets are included. More people recognize the necessity for herbal drug safety monitoring systems. Although arsenic is harmful and carcinogenic, ginseng herbal remedies with arsenic trioxide, or As<sub>2</sub>O<sub>3</sub>, are popular. Traditional Chinese medicine uses arsenic to cure a range of diseases. Despite a lack of research on adverse drug reactions, some commercial bhasmas are metal nanocarriers for health. Not all herbal products do this. Therefore, pharmacovigilance is needed before a herbal treatment is safe for humans. In order to harmonize herbal medicine assessment, the WHO has developed specific guidelines for efficacy, safety, and quality. The UK's Medicines and Healthcare Products Regulatory Agency's "yellow card" program monitors herbal drug safety. Medical herbs are becoming increasingly essential in global health care for treating medical disorders and boosting health since they may be utilized therapeutically. The Canadian Health Care department has reviewed many unapproved Ayurvedic remedies that include excessive lead, mercury, and arsenic amounts in Indian formulations. Some herbal treatments include 0.1 to 0.3 mg of betamethasone, which has corticosteroid-like adverse effects. Drug safety monitoring organizations have reported Ginkgo biloba-related brain hemorrhage, subcutaneous hematomas, delayed prothrombin times, and elevated coagulation times.

### **Techniques in extraction of herbals**

#### **Supercritical fluid extraction (SFE)**

Supercritical fluid extraction (SFE) is the method of choice for removing bioactive compounds from aromatic and medicinal plants. SFE has become a very promising technique for the manufacturing of nutraceuticals and herbal medicines with potent active components. In order to separate the necessary phytoconstituents from the herbal extracts, SFE procedures have been proven to be helpful.

#### **Microwave-assisted extraction (MAE)**

MAE technology can extract phytonutrients, functional and nutraceutical food components, and medicinal actives from biomass. MAE may also extract important fatty acids from oilseeds and microalgae, taxanes from taxus biomass, phytosterols from medicinal plants, polyphenols from green tea, and carotenoids from single cells in addition to inexpensive herbal extracts. This approach has certain advantages over solvent extraction: better product; purity of crude extracts; stability of marker compounds; and safer solvents. b) reduced processing costs, increased marker chemical recovery and purity, fast extraction, and lower energy and solvent consumption.

#### **Solid phase extraction (SPE)**

Analytes are isolated from liquid matrices and purified herbal extracts using the SPE method. In comparison to more conventional sample preparation methods, this technique offers several benefits, including high analyte recoveries, concentration of analytes, highly purified extracts, the capacity to extract analytes of a wide polarity range simultaneously, ease of automation, compatibility with instrumental analysis, and a reduction in organic solvent.

In order to identify thirteen organochlorine pesticide residues in *Scutellaria baicalensis*, *Salvia miltiorrhiza*, *Belamcanda chinensis*, *Paeoniae lactiflora*, *Arisaema erubescens*, *Fructus arctii*, *Anemarrhena asphodeloides*, and *Platycodon grandiflorum*, solid-phase extraction was introduced. Using a combination of acetone and n-hexane solvents, the organochlorine pesticides were extracted from herbs using an ultrasonic method and then removed using a Florisil solid-phase

extraction column. The test solution for the analysis of aristolochic acid I and II in herbal medicines was made using solid phase extraction.

### **Techniques in herbal drug identification and characterization**

#### **HPLC**

The pharmaceutical sector uses analytical and preparative HPLC extensively for the separation and purification of herbal components. Preparative HPLC may be broadly divided into two categories: high pressure HPLC (pressure more than 20 bar) and low pressure HPLC (usually under 5 bar). In preparative HPLC, the quantity of chemical that can be generated per unit time, or throughput, as well as the degree of solute purity are key characteristics to take into account. In analytical HPLC, these include resolution, sensitivity, and rapid analysis time. Using high-performance liquid chromatography (HPLC), the amount of vasicine, the primary bioactive alkaloid of *Adhatoda vusica*, was determined in two polyherbal medicine formulations: Shereeshadi Kashaya (18.1 mg/100 g) and Yastyadivati (0.7 mg/100 g). Sennoside content, kaempferol 3-O-D-gentiobioside, aloe-emodine 8-O-D-glucopyranoside, rhein 8-O-D-glucopyranoside, torachryson 8-O-D-glucopyranoside, and isorhamnetine 3-O-D-gentiobioside L 97 were all determined by HPLC analysis of Senna leaves. Using the RP18 column and an acidic mobile phase, the HPLC technique has reported standardizing the Triphala (an antioxidant-rich herbal formulation) combination of *Emblica officinalis*, *Terminalia chebula*, and *T. belerica* in equal amounts. Currently, the most effective method for quality monitoring of Chinese herbal medication Gan-Cao (licorice) is the combination of HPLC and LC/MS.

#### **High performance thin layer chromatography (HPTLC)**

TLC fingerprinting is the most common herbal analysis method. Resin TLC makes four natural treatments easy to detect. This approach may verify the authenticity of ginseng and *Radix Puerariae* species and evaluate the consistency and stability of their preparations from various manufacturers<sup>101</sup>. However, Di et al. built a fungal polysaccharide acid hydrolyzate fingerprint via automated repeated development. HPTLC fingerprints are typically employed for low- to moderate-polarity chemicals.

Drug companies utilize HPTLC for process development, adulterant detection in herbal goods, pesticide content identification, mycotoxin identification, and quality control of herbs and health foods. HPTLC was used to quantify beta-sitosterol-d-glucoside and Withaferin A in four ashwagandha formulations.

*Syzygium jambolanum* was tested for stability, repeatability, accuracy, and phytoconstituents such as tannin, ellagic acid, gallic acid, and glycoside (jamboline) using HPTLC. To identify, monitor, and quantify bacosides A and B in *Bacopa monnieri* and their formulations, 106 HPTLC was utilized. HPTLC estimated urine THC concentrations to standardize *Cannabis sativa*. HPTLC<sup>108</sup> assessed Withaferin A in herbal extract and polyherbal formulations of *Withania somnifera*. HPTLC can quantify swetiamarin in commercial polyherbal formulations and fresh, large, and little *E. littorale*<sup>109</sup> fruit cultivars. Organoleptic investigation, physico-chemical analysis, TLC, and HPTLC standardized chandanasava, which treats karsya (malnutrition).

Ultra-performance liquid chromatography was used to compare dispensing granule decoctions with regular decoctions for chemical alterations and uniformity.

Chromatographic fingerprinting and metabolomics assist in the control of traditional Chinese medicines (TCMs) quality and operation. The comprehensive study of chromatographic fingerprinting and multivariate analysis approaches in chemometrics and bioinformatics enhanced TCMs' quality and mechanisms of action.

#### **Metabolomics technique**

The identification of active phytoconstituents in herbal medicine has been done using this approach. The chemical components of *Sophora flavescens* were identified using a metabolic method, and their impact on Cytochrome P3A regulation and Pregane X receptor activation was then examined. There have been reports of the increased potential of metabolomics in the discovery of new or better phytotherapeutic drugs via the identification of active secondary metabolites from medicinal plants<sup>134, 135</sup>. Recent research has shown that the purity of a herbal remedy may be determined by combining an orthogonal projection to latent structure-discriminant analysis with an NMR-based metabolomics technique.

### Thermal analysis of herbal drugs

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### Infrared spectroscopy

FTIR along with the statistical method principal component analysis (PCA) was applied to identify and discriminate herbal medicines for quality control in the fingerprint region  $400\text{-}2000\text{ cm}^{-1}$ . The ratio of the areas of any two marked characteristic peaks was found to be nearly consistent for the same plant from different regions, thereby, an additional discrimination method for herbal medicines. PCA clusters herbal medicines into different groups, clearly showing that IR method can adequately discriminate different herbal medicines using FTIR data. Near-infrared spectroscopy technique has been used for rapid determination of active components, species, geographic origin, special medicinal formula, on-line quality control, identification of counterfeit and discrimination of geographical origins of Chinese herbal medicines. Two-dimensional near-infrared (NIR) correlation spectroscopy was applied to the discrimination of *Fructus lycii* (a traditional Chinese medicinal herb) of four different geographic regions.

## II. CONCLUSION

Standardizing herbal remedies includes complete data and controls to practically assure a consistent composition, including marker identification, active principle test, and identification. No law regulates medicinal plants. Various governments have licensing, dispensing, manufacturing, and trading rules to ensure the safety, efficacy, and quality of medicinal plants and their products. Herbal medicine fingerprinting verifies the quality and authenticity of herbal products. The best tools for monitoring traditional herbal medicine quality are chemical fingerprints obtained using spectroscopic, capillary electrophoresis, thermogravimetric analysis, chromatographic, and polarography techniques. All herbal product manufacturers must follow WHO quality standards. Additionally, combining quantitative multicomponent analysis with qualitative fingerprinting provides a novel and reasonable solution to herbal medicine quality control issues. Analytical methods will speed up herbal research and allow companies to set quality standards and seek for regulatory permission to commercialize safe, effective, and shelf-life-compliant herbal drugs. Herbal research will employ advanced hyphenated methods with a technical emphasis to help the pharmaceutical industry quickly and clearly.

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