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REVIEW ARTICLE

Advances in analytical techniques for phytochemical identification and quality control: A comprehensive review

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Abstract

Phytochemistry, the study of plant chemicals, has been a longstanding tradition since ancient times, when plants were used as medicines. Early scientists, such as Dioscorides and Theophrastus, made significant contributions to its development and in the 20th century, the introduction of advanced technologies led to substantial progress in this field. Herbal medicine has gained popularity following the advent of technologies such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, which enable the identification and characterization of bioactive ingredients or phytochemicals. Scientific study is crucial for the authenticity, purity and efficacy of herbal medicines, particularly in light of the growing global demand for them. Herbal medication development follows standardized processes and principles, from plant identification to pharmacological testing. Quality control entails a thorough evaluation that includes identity, validity, physical and chemical characteristics, detection of adulterants and detection of contamination. The World Health Organisation (WHO) and Pharmacopoeias contribute to global standards for herbal drugs, where novel and emerging tools, such as: High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Deoxyribonucleic Acid (DNA) barcoding and several other advanced techniques, aid in the process. This paper focuses on highlighting some of these contemporary analytical procedures that enable researchers to meet the professional requirements for developing herbal medications. Modern quality control in phytochemistry employs advanced methods, including thermal and chromatographic analysis. These techniques help identify plant chemicals more accurately, making it easier to develop and deliver effective medicines. This paper also provides practical insights for professionals in industries related to herbal medicines, natural products and manufacturers of phytochemical-based products, helping them stay informed about the latest technologies. Moreover, it will help develop guidelines and standards for the quality control and safety assessment of phytochemicals, supporting policymakers and regulatory bodies in making informed and updated decisions regarding the use and marketing of phytochemical products.

Keywords: contaminants; drugs; HPLC; phytochemicals; phytochemistry.

Introduction

Phytochemistry, the exploration of plant chemistry and bioactive compounds, boasts a long and diverse history spanning centuries, including the Chinese and Egyptians, who harnessed the medicinal potential of plants, acknowledging the therapeutic attributes inherent in specific herbs and botanicals. An essential aspect of phytochemical examination, involving the recognition and characterization of the chemical constituents present in plant extracts or natural products, is the chemical delineation of phytochemicals. The therapeutic qualities and health benefits associated with medicinal plants are primarily attributed to phytochemicals, which are bioactive substances. The chemical characterization of phytochemicals serves several crucial functions. It aids in establishing the presence of specific bioactive components, understanding the chemical complexity and

composition of plant extracts and evaluating the efficacy, consistency and purity of phytochemical-based products (1). In medicinally important plants, the application of thermal analysis techniques has become indispensable for researchers and scientists seeking a comprehensive understanding of the thermodynamic properties and transformations of natural products. The thermal analysis of these plants involves studying the heat-induced changes in their structure and composition (2). This branch of analysis encompasses various techniques, such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and thermal gravimetric-differential thermal analysis (TG-DTA) (3, 4). These methods enable researchers to analyze the thermal transitions, breakdown patterns and phase shifts of medicinal plants and their associated phytochemicals at various temperatures and meteorological conditions. Pharmaceutical and nutraceutical enterprises must understand the heat stability of medicinal plants and phytochemicals, which aids in improved extraction, protection of bioactive ingredients and product purity and efficacy. The thermal research also demonstrates how temperature affects the therapeutic properties of these plants, helping to retain their efficacy throughout storage (5).

The 20th century witnessed a revolution in phytochemistry, driven by the development of chemical methods and equipment, which facilitated faster extraction, isolation and purification techniques. These advancements were crucial in identifying a diverse range of bioactive substances (6). The structural elucidation of phytochemicals has seen advancements with the introduction of spectroscopic methods, such as Infrared Spectroscopy (IR), Ultraviolet-Visible Spectroscopy (UV-Vis) and Nuclear Magnetic Resonance Spectroscopy (NMR) (7). Chromatographic techniques, including high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC), facilitated the isolation and quantification of plant elements (8). Electrospray ionization (ESI) and air pressure chemical ionization (APCI), two recent developments in mass spectrometry (MS), have made it easier to identify and characterize phytochemicals at the molecular level (9).

Techniques such as HP-TLC have also enhanced resolution and separation, facilitating the identification and quantification of individual phytochemical compounds. Using various stationary phases, mobile phases and detection techniques, HP-TLC can proficiently separate and visualize different phytochemical classes, including alkaloids, polyphenols, terpenoids and aromatic compounds (10). On the other hand, HPLC can handle complex plant extracts, raw materials and finished goods. It possesses exceptional separation qualities, enabling the resolution of complex mixtures and the identification of specific phytochemical components (11). Supercritical fluid chromatography (SFC) has garnered considerable interest and recognition in phytochemical quality control due to its unique advantages and capabilities. One of the primary benefits of SFC in phytochemical analysis is its capability to effectively separate a range of compounds, encompassing both polar and non-polar analytes. Variable selectivity and solvating power can be achieved by utilizing a supercritical fluid, such as carbon dioxide (CO_2), by varying temperature, pressure and co-solvent content. Supercritical fluid chromatography is a state-of-the-art option for separating complex mixtures of phytochemicals due to its versatility, which provides high-resolution separations while minimizing matrix interferences (12).

Particularly for volatile and semi-volatile substances, GC-MS is an excellent choice. Gas chromatography (GC) and MS work together to separate complicated mixtures and identify specific substances based on their mass spectra (13). The detection and quantification of trace components in phytochemical samples are made possible by the highly sensitive and specific analysis provided by GC-MS (14). It is frequently used to examine terpenes, essential oils and other volatile substances found in plant materials (15-17). Liquid chromatography-mass spectrometry is more adaptable and versatile in handling various compound classes, including both polar and non-polar compounds (18). While MS provides precise mass determination and structural elucidation of substances, liquid chromatography (LC) delivers effective separation (19). Liquid chromatography-mass spectrometry is particularly useful for detecting low concentrations of non-volatile substances, such as flavonoids, alkaloids and phenolics, which are commonly found in many plant extracts. Its high sensitivity enables the identification and measurement of phytochemicals in low quantities (20). An essential instrument for phytochemical quality control, inductively coupled plasma-mass spectroscopy (ICP-MS) enables the precise and effective measurement of trace elements and heavy metals (HMs) (21). These techniques provide detailed information on compound compositions, concentrations and the presence of contaminants or impurities. Confirming the identity of the compounds under investigation is made easier by comparing experimental data with recognized reference standards, such as authentic compounds or spectral libraries (22). Advancements in scientific instrumentation, computing capabilities and data analysis methodologies have led to the development of modern analytical techniques for quality control and the identification of phytochemicals (23).

The motivation behind writing this paper is to share the latest advancements in analytical techniques for quality control and to disseminate information on modern methods to a broader audience, including researchers, academics and professionals in the field of quality control and chemical identification of phytochemicals. This will contribute to the scientific community by fostering discussions and advancements, providing insights into how these techniques can be applied to verify the authenticity, purity and safety of phytochemical products and encouraging interdisciplinary research by showcasing the integration of modern analytical methods in the study of phytochemicals.

Significance of quality control of medicinal plants

Herbal medications require strict quality control to ensure their safety, potency, purity and consistency. It also entails inspecting the compound or medicine for the presence of adulterants and determining its hazardous/damaging effects on living systems (24). With rising global interest in traditional medicine, there is an increasing demand for medicinal plants, necessitating robust quality control measures. Furthermore, quality control extends to the identification and screening of contaminants, including HMs, pesticides and microbial agents, reinforcing the commitment to maintaining the highest standards in herbal medicine production (24). Quality control addresses this by evaluating plant identity, purity and potency (25). Accurate species identification is crucial, as misidentification can lead to contamination or toxicity (26). DNA barcoding enables precise species authentication by analyzing specific DNA regions (Fig. 1) (27). A study using 1,452 rbcL and matK barcodes from 521 herbs achieved species

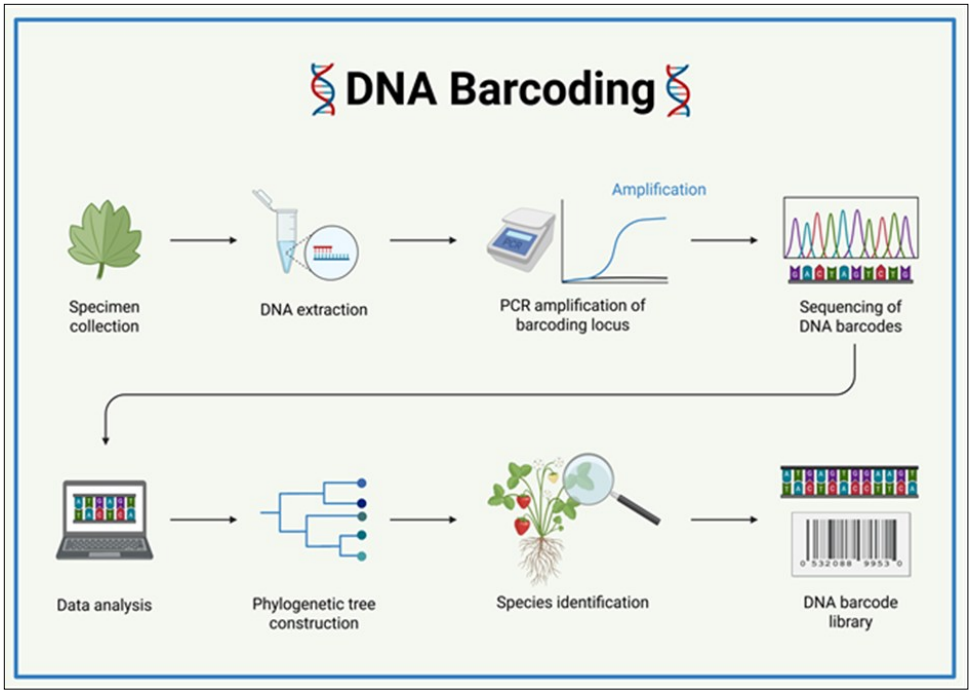

Fig. 1. Workflow of DNA barcoding.

resolution rates of 74.4 % %, 90.2 % % and 93.0 % %, respectively, revealing adulteration in 20 % % of samples, 6 % % of which had toxic properties (27). The resulting barcode library supports routine drug authentication for stakeholders (27).

The variability in growing, storage and processing conditions frequently increases the presence of toxic chemicals, microbial load, contaminants and other undesirable elements in medicinal plant raw materials, making purity essential (28). It is thus imperative to detect and monitor these levels meticulously, to ensure the safety and quality of the medicinal herbs Fig. 2 illustrates the phytochemical quality control process in human health. Overall, DNA barcoding, chromatography and spectroscopy are crucial for ensuring the authenticity, purity and potency of herbal medicines, key to their safe global use.

Development of herbal medicine

Stages in the development of herbal medicines

The first step in developing an herbal medicine is collecting the desired plant material, followed by a proper evaluation of its identity and authenticity (29). The collected plant is then processed systematically, adhering to standardized parameters. Accurate identification involves taxonomy, nomenclature and family classification, typically verified by experts or recognized authorities, such as herbaria (30). Chemical evaluation helps determine the purity and presence of key plant therapeutic constituents. It includes measuring extractive yield, selecting suitable extraction methods and conducting preliminary phytochemical screening (31). Recent advances utilize marker compounds in chemical fingerprinting to assess the potency of crude materials. This technique identifies characteristic patterns of chemical markers-categorized as bioactive, therapeutic, principal, or toxic components. Chemical fingerprinting enhances quality control by validating the authenticity and detecting adulterants, thereby ensuring the efficacy and safety of herbal products (32). Fig. 3 provides a general outline of the steps involved in standardizing and assuring the quality of medicinal plants, while Fig. 4 depicts the step-by-step process for authenticating plant materials when developing herbal medicines.

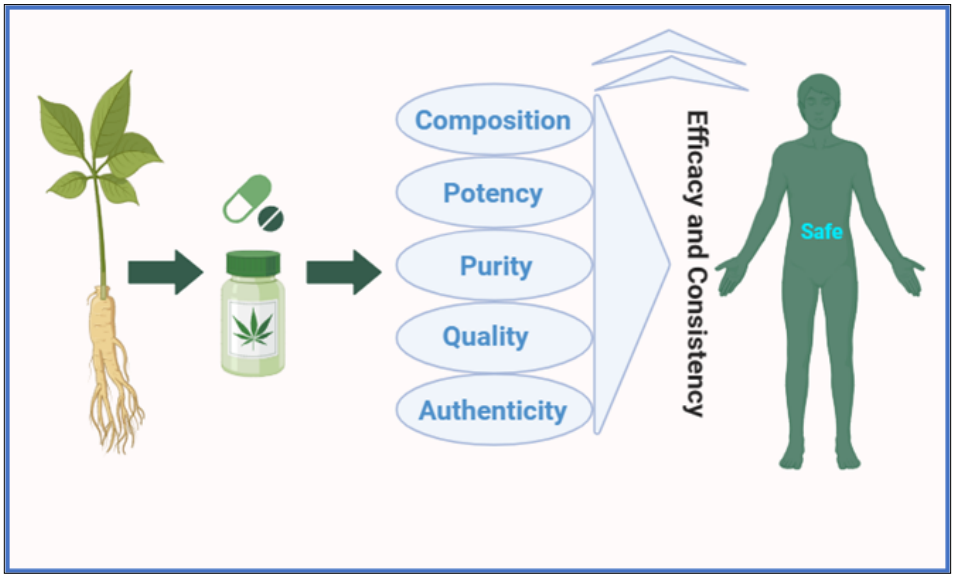

Fig. 2. Blueprint of the routine of quality control.

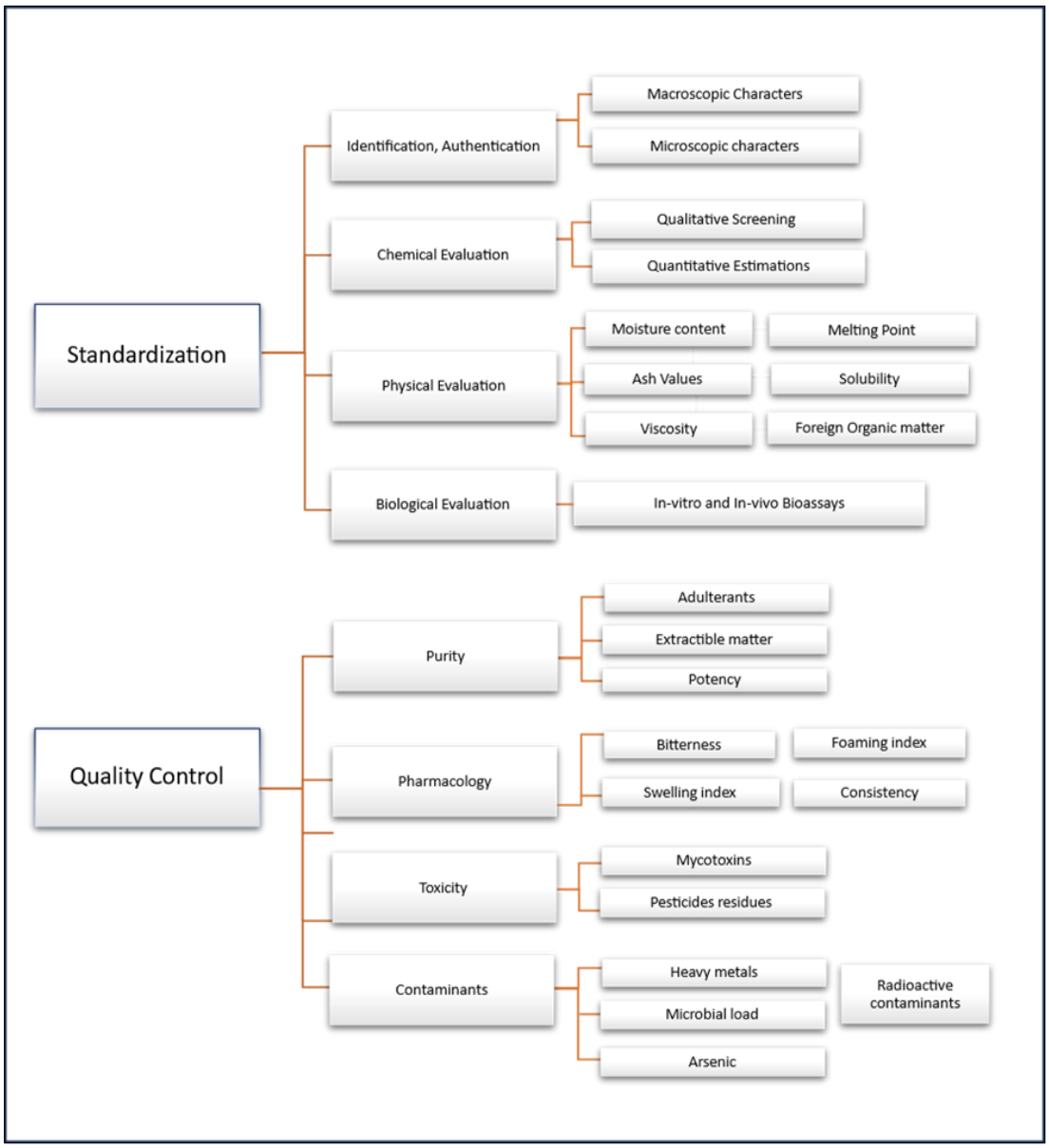

Fig. 3. Outline the methods involved in the standardization and quality assurance of medicinal plants.

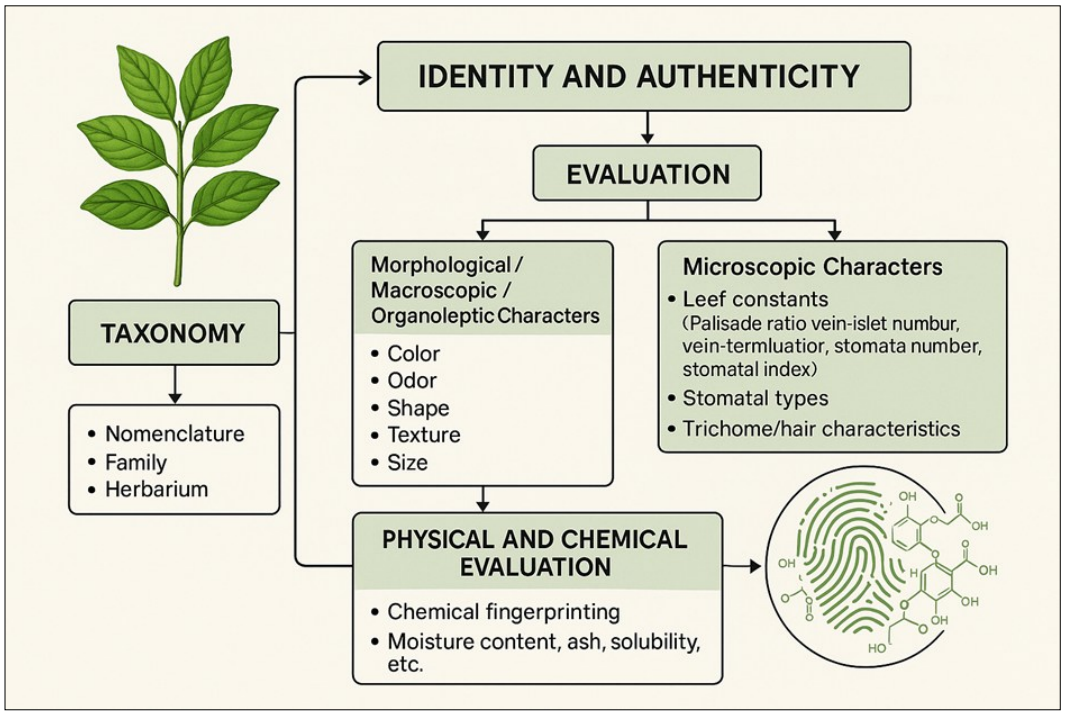

Fig. 4. Schematic diagram showing different aspects of identification and authentication of a plant sample.

Tools and techniques used in standardization, quality assurance and control of herbal medicines

Thermal analysis of medicinal plants and phytochemicals: Medicinal plants have been harnessed for their therapeutic potential. In this context, understanding the thermal behavior of their bioactive constituents is crucial for pharmaceutical applications (33). In this section, the various thermal analysis techniques will be discussed.

Differential scanning calorimetry

Differential scanning calorimetry is a thermal analysis tool employed to quantify the heat flow linked with physical and chemical transformations in a substance relative to temperature changes, crystalline structure and phase transitions (34, 35). The main components of a DSC instrument include a sample pan, a reference pan (often an empty pan), a heating element and a temperature controller (Fig. 5) (36). In a standard DSC experiment, the sample and reference pans undergo identical temperature changes and any heat taken up or released by the sample is compared with that of the reference. The temperature variance between the sample and reference is documented as a time- or temperature-dependent variable (37). A study aimed to enhance the gastrointestinal absorption of *Ginkgo biloba* extract (GBE) by creating phospholipid complexes (GBP) and solid dispersions (GBS). The physicochemical properties of the compounds mentioned above were assessed using DSC. Subsequent pharmacokinetic assessments in rats demonstrated a significant enhancement in the oral bioavailability of various flavonoids, including quercetin, when administered in GBP and GBS compared to GBE. Notably, the bioavailability of GBP exhibited a substantial increase compared to GBS, resulting in improved bioavailability (38).

Thermogravimetric analysis

Thermogravimetric analysis is another thermal analysis method used to examine how a material's weight changes with temperature or time under controlled conditions. It is particularly valuable for assessing the thermal stability, composition and decomposition kinetics of diverse substances (39). In a TGA experiment, the sample and a reference material, often an empty pan, are heated simultaneously and the alteration in the sample's weight is continuously monitored. The temperature is systematically increased and the TGA instrument records the sample's weight loss or gain as it undergoes thermal processes (40). This technique is widely used in research and industry to gain insights into the thermal characteristics of materials and their responses to varying temperatures. A study focused on enhancing the aqueous solubility and bioavailability of *Boswellia serrata* Roxb. extract (BSE), renowned for its anti-inflammatory properties, by incorporating it into a lamellar solid hydrotalcite-like anionic clay (41). The ionic lamellar compound, chosen for its layered crystal structure, was expected to encapsulate boswellic acids, the active components of BSE. The structure was calcined to enhance the loading of organic acids. The resulting hybrid composites were extensively characterized using TGA to understand their characteristics, interplay and estimation of the active molecules within the materials loaded with plant extract (41).

Thermal gravimetric-differential thermal analysis

Thermal Gravimetric-Differential Thermal Analysis is a combined thermal analysis technique that integrates the principles of TGA and DTA, used to simultaneously measure a sample's weight changes (TGA) and temperature (DTA) as it undergoes thermal processes (42). The main components of a TG-DTA instrument include a sample pan, a reference pan, a balance for weight measurements (TGA) and a thermocouple for temperature measurements (DTA). The sample and reference pans are heated simultaneously and the balance records any weight loss or gain of the sample, while the temperature probe notes the temperature variance between the pans (43). A study analyzed the constituents and lipid profile of *Caryocar villosum* oil and assessed its heart health benefits, thermogravimetric-differential, calorimetric and spectroscopic behavior (44)

Dynamic mechanical analysis (DMA)

Dynamic mechanical analysis is a versatile and sophisticated method used to assess the mechanical characteristics of materials in relation to temperature, time, frequency, or a combination of

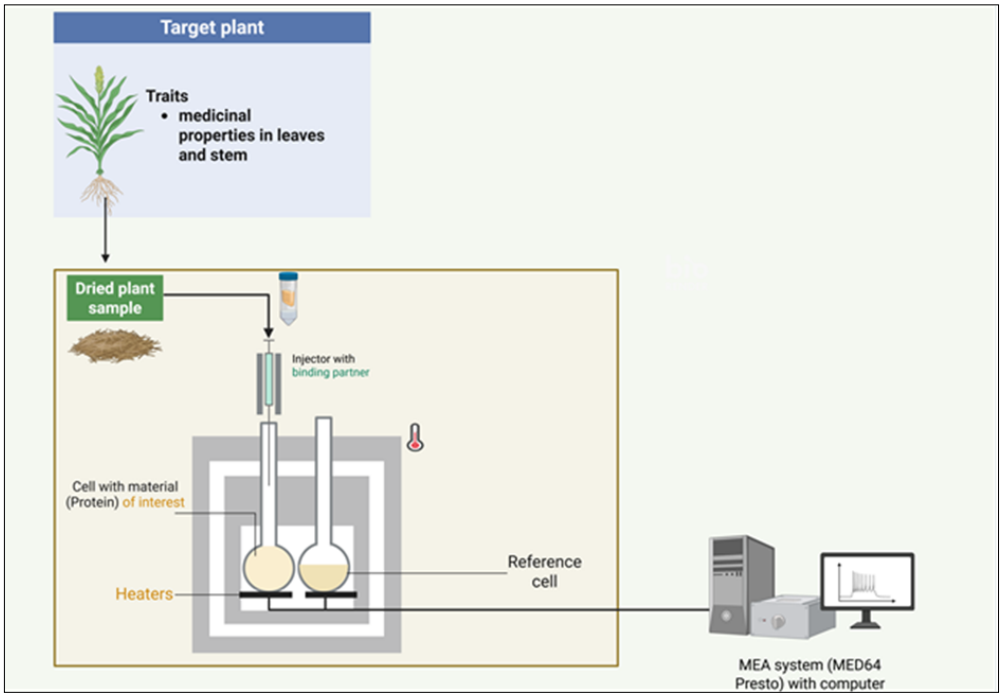

Fig. 5. Workflow of differential scanning calorimetry.

these factors (45). DMA is particularly valuable in characterizing the viscoelastic behavior of materials, providing insights into their dynamic mechanical response under various conditions (46). A study focused on enhancing the overall stability of natural fiber composites (NFCs), which are recognized for their environmentally beneficial properties, including biodegradability. The research introduces three novel fibers and fillers-*Morinda tinctoria* wood powder and myrobalan-aiming to enhance the performance of biodegradable hybrid composites (47).

Chromatographic analysis

The chromatographic profiles mostly mirror the sample's chemical composition, as observed through the selected detection techniques. The content of this substance relies heavily on determiners such as the specific species, plant component, ambient circumstances and extraction process (31). In chromatographic analysis, the quality and degree of authenticity of herbal preparations are assessed by utilizing one or two marker molecules or pharmacologically active constituents found in the mixtures (48). Since most recent state-of-the-art facilities generate massive amounts of data, which can be challenging to process, the relevance of data-processing techniques that utilize chemometrics is instrumental. Chemometrics utilizes statistics and mathematical concepts to analyze data, thereby optimizing the collection and application of valuable knowledge (49). The efficacy of chemometric techniques in facilitating multidimensional calibration of specific spectroscopic, electrochemical and chromatographic procedures is exemplified. The primary application of this approach is to interpret UV-Vis and near-IR (NIR) spectra, as well as data obtained from other instrumental methods, which enables the characterization and quantitative analysis of active substances in complex mixtures, particularly in the study of medicinal preparations available on the market (49).

High-performance thin-layer chromatography analysis

Currently, one of the most effective analytical methods for analysing phytochemicals is HPTLC, which offers superior separation efficiency when identifying and detecting complex biomolecules in plant extracts used in botanical research and herbal medicines (50). The benefits of HP-TLC are scanning, optimization, automation, computerization, selective detection and minimal sample preparation (51). Table 1 presents selected studies that utilize HPTLC for detecting marker molecules and evaluating the quality of herbal formulations.

High-performance liquid chromatography analysis

High-performance liquid chromatography is a user-friendly, highly automated and widely used technique for the quality assessment and standardization of herbal preparations, due to its capability to identify the presence of markers in both qualitative and quantitative manners (57). An important advantage of HPLC is the ability to perform hyphenation with various detecting molecules like diode array detection (DAD), fluorescence detection (FD), evaporative light scattering detection (ELSD), MS and computer-aided design (CAD) (58-62). The working procedure of HPLC is depicted in Fig. 6, while its applications in assessing polyherbal formulations and herbal preparations are illustrated in Table 2.

Liquid chromatography mass spectrometry (LC-MS) analysis

High performance liquid chromatography alone has limitations in analyzing complex raw extracts, which are overcome by integrating LC-MS, enabling enhanced accuracy through combined separation and mass detection. Liquid chromatography mass spectrometry offers a discriminating method that enables the measurement of both quantity and structure and can attain very low sensitivities of picograms per milliliter (68). The LC-MS-ion trap mass spectrometry method can be simplified by coupling it with quadrupole time-of-flight high-resolution mass spectrometry (Q-TOF HRMS) and triple-quadrupole mass spectrometry (TQ-MS), alternative techniques that can be used in conjunction with the HPLC analytical approach (69-71). The LC-MS approach possesses unique capabilities, such as structure characterization, determination of molecular mass, fragmentation information,

Table 1. Evaluation of selected polyherbal formulations and plant-based drugs by HPTLC

Name of the plant material/formulation/ drug	Major constituents	Pharmacological properties	Important compounds detected	Major outcome of the investigation	Reference
Sanwujiao pills	Polygoni Multiflori Radix, Aconiti Radix, Aconiti Lateralis Radix Praeparata, Aconiti Vilmoriniani Radix, Olibanum, Typhonii Rhizoma	Treatment of rheumatism and blood stasis	Emodin, Physcion, 2,3,5,4′-tetrahydroxy stilbene-2-O-β-D-glucoside, Benzoylaconine, Benzolymesaconine, β-Boswellic acid and 3-acetyl-β-boswellic acid	Qualitative analysis of intricate herbal preparations and detection of phytochemicals.	(52)
Divya-swasari-vati	*Glycyrrhiza glabra*, *Syzygium aromaticum*, *Cinnamomum zeylanicum*, *Pistacia integerrima*, *Cressa cretica*, *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Anacyclus pyrethrum*	Treatment of pulmonary ailments	Gallic acid, Glycyrrhizin and Eugenol	The swift standardization of Divya-Swasari-Vati.	(53)
Jatyadi taila	*Curcuma longa*, *Terminalia chebula and Jasminum officinale*	Antiseptic, treatment of wounds	Curcumin, Gallic acid and Ursolic acid	Standardized method for quality control and determination of stability of the marker compounds.	(54)
Mahasudarshan Churna	*Swertia chirata*	Treatment of fever, cold and malaria	Oleanolic acid, Mangiferin, Gallic acid, Ursolic acid, Curcumin, Quercetin	An economical, rapid, precise and accurate method to measure the marker chemicals in the formulations.	(55)
Centella asiatica and its commercial products	*Centella asiatica*	Treatment of the nervous system	Asiaticoside	Quantification of Asiaticoside in the formulation.	(56)

Table 2. Evaluation of selected polyherbal formulations and plant-based drugs by HPLC

Name of the plant material/formulation/ drug	Major constituents	Pharmacological properties	Important compounds detected	Major outcome of the investigation	Reference
Brahmi vati	*Bacopa monnieri* L. and *Piper longum* L.	Treatment of epilepsy	Bacoside A_3 and Piperine	Substantial increase in bacoside A_3 and piperine in the in-house sample compared to all three marketed samples.	(63)
Trichup tablet	*Eclipta alba*, *Glycyrrhiza glabra*, *Emblica officinalis*, *Centella asiatica*, *Hibiscus rosa-sinensis*, *Tinospora cordifolia*, *Tribulus terrestris*, *Powder of Triphala Churna* and *Shukti Bhasma*	Treatment of hair and scalp	Gallic acid	Quick and accurate estimation of gallic acid in the sample.	(64)
Qurs-e-Gul and crude drug	*Glycyrrhiza glabra*, *Pistacia lentiseus*, *Bamboosa bamboo*, *Nardostachys jatamansi*, *Rosa damascene*	Treating jaundice and cardiac ailments	Glabridin	Accurate estimation of glabridin in the sample.	(65)
Ashwagandhadi lehyam	*Withania somnifera*, *Hemidesmus indicus*, *Cuminum cyminum*, *Smilax china*, *Vitis vinifera*, *Elettaria cardamomum*	Used as a tonic, hypnotic, sedative and diuretic	Withaferin-A	The Ashwagandha lehyam formulation has a concentration of 0.092 % % of withaferin-A.	(66)
Pathyashadangam kwath	*Terminalia chebula*, *Terminalia bellirica*, *Phyllanthus emblica*, *Andrographis paniculata*, *Tinospora cordifolia*, *Curcuma longa*, *Azadirachta indica*	Treatment of diseases of the eye, ear and tooth, migraine and cluster headache	Gallic acid	Quantification of the gallic acid in three batches of the sample (16.14 mg/mL, 23.22 mg/mL and 19.29 mg/mL) of kwath, respectively.	(67)

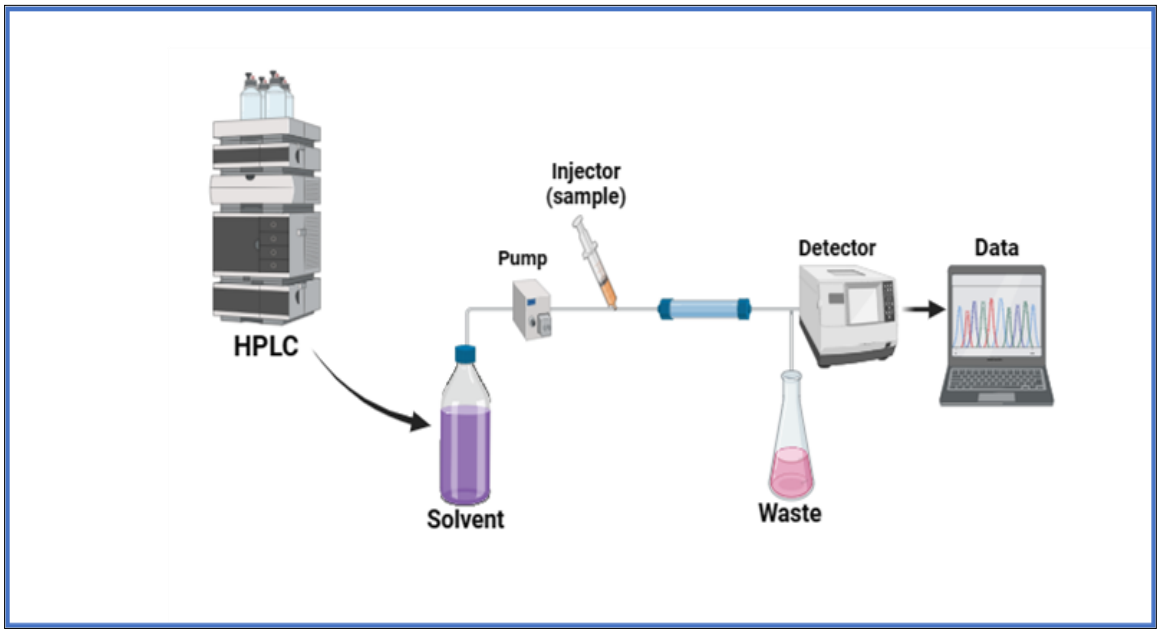

Fig. 6. Workflow of high-performance liquid chromatography.

retention time, a broad detection range and excellent separation of analytical substances that are not present in HPLC alone (72-75). The working principle of LC-MS is represented in Fig. 7, while Table 3 illustrates the identification and quality evaluation of raw plant products and polyherbal formulations using the technique.

Gas chromatography-mass spectrometry analysis

Gas chromatography is used to measure the concentration of volatile compounds in plant matrices (81). The benefits of GC include high sensitivity, low sample requirements, robust separation capabilities, excellent selectivity, broad applicability and rapid analysis (82). Gas chromatography separates mixture components based on their interactions with a stationary phase and a mobile carrier gas. Variations in the compounds' structures and affinities cause them to elute at different times under the same driving force (82). The motivation behind coupling a GC (83) with an MS was to combine the complementary features of these two analytical tools. The GC separated components of mixtures into distinct pure compounds, which eluted progressively. On the contrary, the MS offered a strong approach to detecting previously undiscovered pure compounds (84). Gas chromatography-mass spectrometry is a method that combines two analytical techniques to detect and characterize chemicals found in a plant sample. Gas chromatography-mass spectrometry is a valuable tool for analyzing phytochemicals and studying chemotaxonomy in medicinal plants, as it enables the generation of high-quality chemical fingerprints using capillary columns (85). Additionally, GC-MS provides qualitative and semi-quantitative data through mass spectral databases, aiding in research into the pharmacological relevance of herbal constituents (86). The working mechanism of GC-MS is illustrated in Fig. 8, while its application in the quality evaluation and standardization of herbal medicine is exemplified in Table 4.

Supercritical fluid chromatography analysis

Supercritical fluid chromatography (SFC) utilizes a low-viscosity supercritical fluid, typically CO_2, as the mobile phase to dissolve primarily nonpolar compounds (92). By adding cosolvents like methanol or ethanol, SFC can also analyze polar analytes, offering superior separation efficiency and faster analysis than LC and effectively separating heat-sensitive molecules, unlike GC (93). Its lower use of organic solvents makes it more environmentally friendly and its low viscosity results in reduced pressure drops compared to traditional LC (94). SFC is a versatile method for separating, quantifying and assessing the quality of both polar and nonpolar natural compounds in herbal products. Table 5 illustrates the application of SFC in the quality assessment of the herbal tenets.

Technique for the analysis of elements

Inductively coupled plasma mass spectrometry (ICP-MS) analysis

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is employed for elemental analysis (28). It is a reliable analytical method for qualitative multi-elemental determination, quantitative concentration measurement and isotopic abundance assessment across diverse matrices. An ICP-MS system typically includes a sample introduction system, an ion source (ICP), electrostatic lenses, an interface, a mass spectrometer and a detector (98). In a standard setup, samples are introduced via an autosampler, then directed by a peristaltic pump to the nebulizer, where they are

Table 3. Evaluation of selected polyherbal formulations and plant-based drugs by LC-MS

Name of the plant material/formulation/ drug	Major constituents	Pharmacological properties	Important compounds detected	Major outcome of the investigation	Reference
Majoon-e-Nisyan	*Boswellia serrate Roxb*., *Acorus calamus* L., *Cyperus rotundus* L., *Piper nigrum* L., *Zingiber officinale* L.	Treatment of amnesia	(α + β) boswellic acid, β-asarone, luteolin, 6-gingerol and piperine	Boswellic acid (α + β), β-asarone, luteolin, 6-gingerol and piperine were quantified as follows: 0.973 ± 0.009, 0.093 ± 0.004, 0.003 ± 0.001, 0.0256 ± 0.002 and 0.0266 ± 0.008 mg/g of the formulation.	(76)
Peedantak Vati	*Balsamodendron mukul*, *Colchicum luteum*, *Withania somnifera*, *Strychnos nux-vomica*, *Cyperus scariosus*, *Pluchea lanceolata*, *Vitex negundo*, *Boerhaavia diffusa*	Treatment of inflammation	Rutin, Caffeic acid, Colchicine, Withaferin A and Curcumin	Confirmation of the presence of marker phytochemicals.	(77)
YANG XIN	*Angelica sinensis*, *Cuscuta chinensis*, *Nelumbo nucifera*, *Dioscorea batatas*, *Cistanche salsa*	Improve cognitive function	4,5 Dicaffeoylquinic acid, Forsythoside A, Ginsenoside R, Sibiricoses A5 and Schisandrol A,	18 analytical markers were identified for YANG XIN formulations.	(78)
Andrographis paniculata Capsules	*Andrographis paniculata*	Treatment of fever and diarrhoea	Andrographolide, Dehydroandrographolide and Neoandrographolide	In *A. paniculata* capsules, the amounts of andrographolide, dehydroandrographolide and neoandrographolide were found to be 3.90-4.08, 4.77-5.04 and 4.32-4.48 mg/g, respectively.	(79)
Hyeonggaeyeongyo-tang	*Schizonepetae spica*, *Forsythiae fructus*, *Saposhnikoviae radix*, *Angelicae gigantis radix*, *Cnidii rhizoma*, *Bupleuri radix*, *Aurantii fructus Immaturus scutellariae radix*, *Angelicae dahuricae radix*, *Platycodonis radix*, *Paeoniae radix*, *Gardeniae fructus and Glycyrrhizae radix et rhizome*	Antioxidant and antimicrobial properties	Gallic acid, Narirutin, Baicalin, Neohesperidin, Paeoniflorin, Naringin, Pulegone, Glycyrrhizin and Wogonoside	The marker compounds were effectively estimated.	(80)

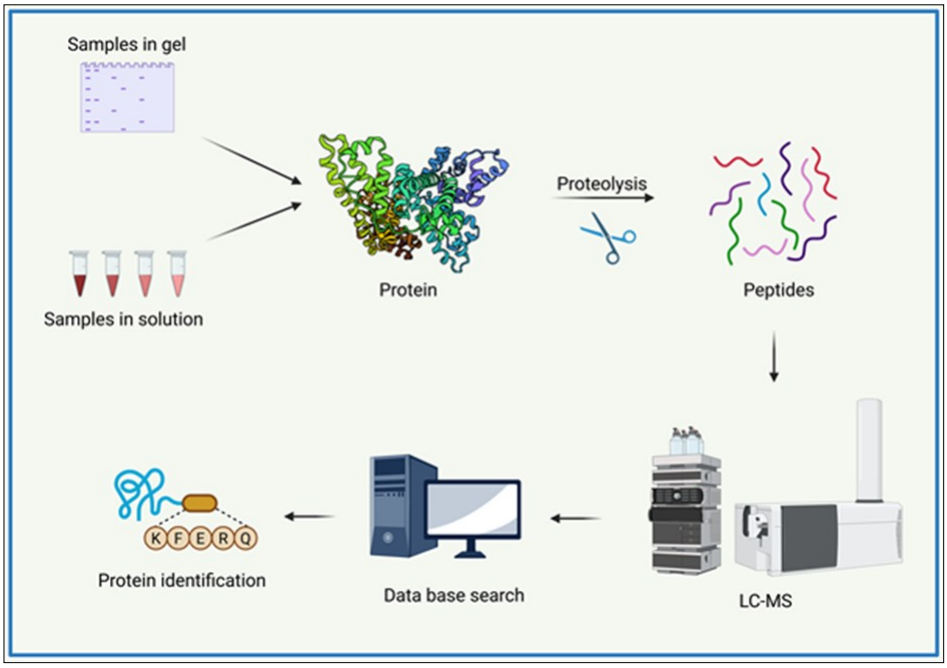

Fig. 7. Workflow of liquid chromatography-mass spectrometry.

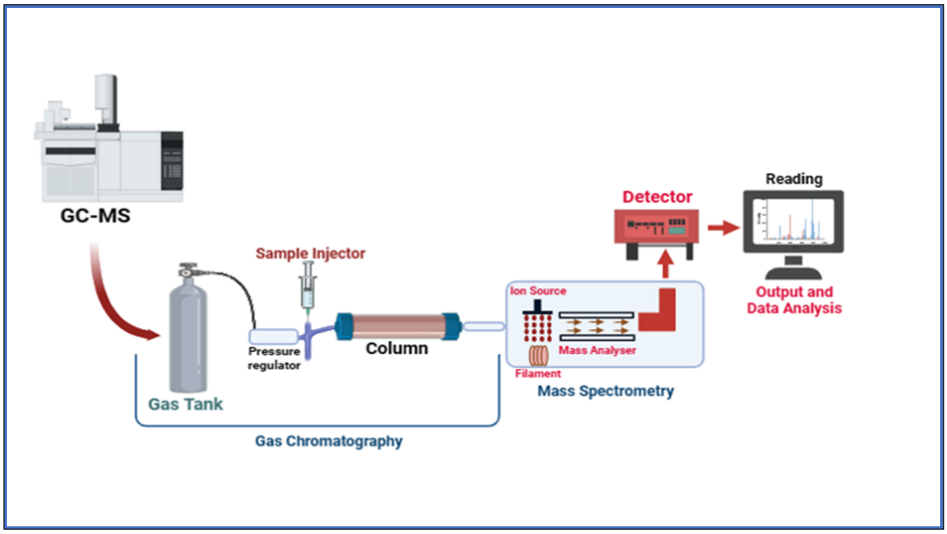

Fig. 8. Workflow of gas chromatography-mass spectrometry.

Table 4. Evaluation of some polyherbal formulations and polyherbal formulations by GC-MS

Name of the plant material/formulation/ drug	Major constituents	Pharmacological properties	Important compounds detected	Major outcome of the investigation	Reference
Succus Bambusae	*Succus Bambusae*	Acts as a natural beverage		Twenty-nine volatile components were initially discovered from a sample of forty batches and six quality markers were identified.	(87)
Clinacanthus nutans	*Clinacanthus nutans*	Treatment of diabetes, inflammatory disease, skin disease, etc.	Squalene, stigmasterol, betulin,	Identification of chemical markers of the plant for future quality evaluation.	(88)
Aswagandharishtam	*Withania somnifera*, *Chlorophytum tuberosum*, *Rubia cordifolia*, *Terminalia chebula*, *Curcuma longa*, *Berberis aristata*, *Glycrrhiza glabra* etc.	Used as a nervine tonic	Prostaglandin A2, cholesterol, piperine, gentamicin a, d-mannose, eugenol and pipradrol	A preliminary report was of indication of the different types of biomolecules contained in Aswagandharishtam.	(89)
Arq-e-Nana (AeN), Arq-e-Gazar (AeG) and Arq-e-Brinjasif (AeB)	*Mentha Arvensis*, *Daucus carota*, *Achillea mellifolium*	Treatment of vomiting, dyspepsia, palpitation, inflammation of the liver and intestine	Limonene, 1,8 cineole, fenchone, camphor, citronellol, terpineol, thymol and carvone	Quality control and stability analysis of Arq formulations from the Unani Pharmacopoeia of India.	(90)
Safoof-e-Pathar Phori	*Didymocarpus pedicellata*, *Dolichos biflorus*, *Rheum emodi*, *Raphanus sativus*	Antiurolithiatic activity	3-furano carboxylicn acid, cyclooctene, linolenic acid, n-hexadecanoic acid and alpha humulene	An authentic tool for quality analysis of the polyherbal formulation.	(91)

Table 5. Application of SFC in the quality assessment of herbal principles

Name of the herbal drug	Pharmacological properties	Important elements detected	Major outcome of the investigation	Reference
Rhodiola rosea	Treatment for stress, fatigue and weakness	p-tyrosol, rosin, rosiridin, salidroside, rosarin, rosavin and tricin-5-O-β-d-glucopyranoside	The established methodology enabled a comprehensive extraction of all seven chemicals using a mixture of 60 % % CO_2 and 40 % % methanol.	(95)
Isatidis Radix (root of *Isatis indigotica*)	Treatment of cold, fever, sore throat, mumps and tonsillitis	(R, S)-goitrin	This endeavor investigated the utilization of SFC in the field of chiral research about traditional Chinese medicine.	(96)
Artemisia annua	Antimalarial activity	Artemisin	The results suggest that SC-CO_2 and SC-CO_2 + ethanol extraction methods show promise as alternatives for extracting artemisinin. These methods avoid the need for organic solvents like hexane and yield extracts suitable for making antimalarial pharmaceuticals.	(97)

combined with argon gas to form an aerosol. The spray chamber eliminates large droplets and controls the liquid flow (99). The fine aerosol is then ionized in high-temperature argon plasma through vaporization, atomization and ionization. The extent of ionization depends on plasma temperature and the ionization potential of elements (100). ICP-MS, utilizing an argon plasma, converts the sample into ions and analyzes them using a mass spectrometer (21). Unlike ICP-OES, which uses optical detection, ICP-MS directly quantifies ions and offers lower detection limits (101). It detects trace elements in ppb or ppt levels and provides advantages such as a wide linear range and isotopic analysis (101). ICP-MS is extensively used to determine in medicinal plants, addressing global health risks when levels exceed threshold limits (102). Fig. 9 illustrates the working principle of ICP-MS, while Table 6 shows the application of ICP-MS in quantifying HMs and assessing the quality of selected herbal samples.

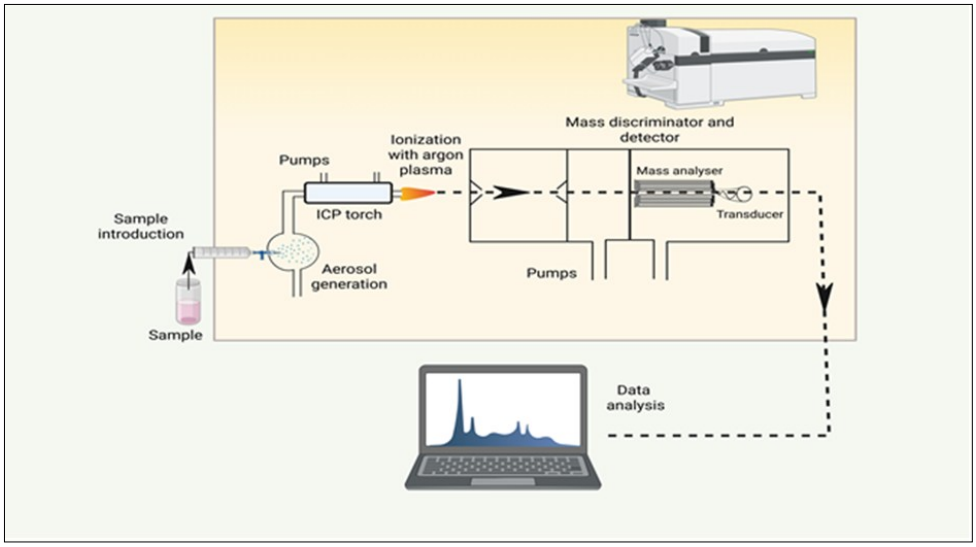

Fig. 9. Workflow of inductively coupled plasma-mass spectrometry.

Table 6. Evaluation of selected herbal formulations or medicinal plants by ICP-MS

Name of the plant material/ formulation/drug	Pharmacological properties	Important elements detected	Investigation outcomes	Reference
Niuhuang Qingwei Pills	Treatment of gastrointestinal problems	Excess of lead (Pb), arsenic (As) and Antimony (Sb)	A set of fundamental procedures for evaluating the potential risks associated with Chinese patent medicine has been developed for the first time.	(103)
Astragalus membranaceus	Improvement function of the immune system	The computed recommended maximum residue limits (MRLs) for Pb, cadmium (Cd), mercury (Hg) and copper (Cu) in the aforementioned medical items were found to be higher than the MRLs established by the International Organization for Standardization (ISO) and the Chinese Pharmacopoeia.	This study employed a pragmatic research methodology to assess the safety of medicinal herbs contaminated with various HMs.	(104)
Ayurvedic dietary supplements	Multifarious health benefits largely boost the immune system	The samples of dietary supplements exhibited Hg levels ranging from 0.002 to 56 µg/g.	An effective way of estimating metallic content in ayurvedic formulations.	(105)
Qishiwei Zhenzhu Pills (Tibetan medicine)	Treatment of stroke, paralysis, hemiplegia, cerebral hemorrhage	The elements in Qishiwei Zhenzhu tablets were ranked in the order of Cu > Hg > Pb, with mass fractions above 6000 µg/kg. The mass fractions of silver, arsenic, manganese, gold, strontium, barium, chromium and nickel ranged from 33 to 1034 µg/kg.	Analysis of trace elements in Qishiwei Zhenzhu Pills.	(106)
Vaiśvānaracūrna Tablet	Treatment of flatulence, duodenal ulcers, rheumatism and cardiac ailments	Presence of Cd, Pb, Cr, As, Cu and Zn within the permissible limits	Effective quality evaluation of the polyherbal formulation.	(107)

Conclusion

The development of modern analytical techniques for quality control and chemical identification of phytochemicals represents a pivotal stride in the convergence and revolution of traditional herbal medicine. It has deciphered the intricate compositions of diverse plant extracts with precision and reliability, assessing the quality and authenticity of phytochemical-based products through enhanced phytochemical analysis, quality control, quantification and evaluation. This ensures industries have powerful tools to ensure product integrity and safety. These modern analytical techniques have helped in isolating new bioactive compounds, formulating herbal medicines and advancing natural product research, particularly through the use of thermal analysis and chromatographic methods. The synergy between chromatography and thermal analysis has facilitated the comprehensive profiling of phytochemical mixtures, aiding in the detection and separation of specific compounds crucial for the expansion of pharmaceuticals, nutraceuticals and other applications. Such steps are essential to quality control because they ensure that herbal goods adhere to legal requirements and are free from harmful substances. Rigorous quality assurance protocols, facilitated by cutting-edge analytical tools, safeguard consumer health and bolster the credibility of phytochemical-based interventions in diverse sectors. Lastly, chemometrics and sophisticated data analysis techniques are essential to quality control. The interpretation of complicated data sets, classification of samples and detection of outliers or adulterants are all made conceivable by using statistical tools, multivariate analysis and pattern recognition techniques. These techniques improve the consistency and precision of quality control procedures. In recent years, artificial intelligence and bioinformatics have enhanced the quality of herbal drug development through in-silico studies, utilizing software for screening phytochemicals and molecular docking to determine their efficacies.

Like in any scientific discipline, there are still issues to be resolved. Among the active areas of study and development in this subject are the complexity of plant matrices, sample preparation, the need for standardization and the use of reference materials. Overall, the multidisciplinary subject of chemical identification of phytochemicals uses analytical methods, spectral analysis and reference standards. It is essential to comprehend the chemical complexity of plants, discover novel bioactive substances, ensure product quality, advance the study of natural products and identify new drugs. As technology advances, these methods will remain essential in releasing the full potential of phytochemicals for various uses, from medicine to agriculture and beyond. In essence, the integration of modern analytical techniques with the rich heritage of traditional herbal wisdom strengthens the foundations of phytochemical research. It propels the field toward new horizons of discovery and application. These findings serve as a testament to the transformative power of analytical innovation in unraveling the secrets of nature's pharmacopoeia, offering a roadmap for future endeavors in harnessing the full extent of phytochemicals beneficial to mankind.

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Authors' contributions

DS conceptualized and supervised the manuscript and participated in the composition of the original draft, internal reviewing and overall coordination. ZK, AM, MD, SG, SD, SSN, AD and RS contributed to the sectional composition, editing and revision of the manuscript. AKM edited the manuscript, provided overall supervision and contributed to the artwork. SB was responsible for quality checking, formatting and revision. NP and SC contributed to the revision and reference management of the manuscript.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly Premium, Quillbot Premium and Chat GPT to remove plagiarism, grammatical, sentence construction and typographical errors. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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