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Review Article

Recent Advances in Standardization and Quality Control of Polyherbal Formulations for Management of Diabetes

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia and is associated with complications affecting multiple organ systems. Interest in medicinal plants and polyherbal formulations has increased because plant-derived phytochemicals may influence glucose metabolism through complementary biological pathways. Polyherbal formulations combine two or more medicinal plants or extracts in defined proportions; however, differences in plant identity, geographical origin, cultivation, harvesting, processing, extraction and storage can alter their composition and quality. This review summarizes approaches used to standardize and control the quality of polyherbal formulations investigated for diabetes management. The review covers authentication of raw herbal materials by botanical, macroscopic, microscopic and molecular methods; physicochemical evaluation; phytochemical screening; marker-based standardization; and testing for contaminants such as heavy metals, pesticide residues, microbes and residual solvents. It also discusses conventional analytical methods and advanced tools, including thin-layer chromatography, high-performance thin-layer chromatography, high-performance liquid chromatography, gas chromatography, chromatographic fingerprinting and metabolomics. Chemometric approaches such as principal component analysis and hierarchical cluster analysis can help compare chemical profiles, identify outliers and assess batch-to-batch consistency. Biological evaluation, including enzyme-inhibition assays, cellular models and appropriate in-vivo studies, may provide complementary evidence of activity and safety. Linking chemical fingerprints with biological findings can strengthen the rationale for selecting quality markers. Overall, no single test test is is s sufficient to charact characterize a complex polyherbal product. A combined, validated quality-control strategy is needed to support identity, purity, consistency and reproducibility.

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Although some formulations have shown promising glycaemic outcomes in studies, laboratory or preclinical findings alone do not establish clinical efficacy. Well-designed clinical trials, transparent manufacturing controls and compliance with relevant pharmacopoeial and regulatory standards remain important for determining safety, effectiveness and dependable use in diabetes management.

INTRODUCTION:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia due to inadequate insulin secretion, impaired insulin action, or both. It is a major global health problem associated with serious complications involving the cardiovascular system, kidneys, eyes and nervous system. The increasing prevalence of diabetes has stimulated interest in complementary and alternative approaches, including herbal medicines.[1] Medicinal plants contain diverse bioactive compounds that may influence glucose metabolism and other pathological processes associated with diabetes. Consequently, herbal medicines have received increasing attention as potential supportive therapies for diabetes management.[2] Polyherbal formulations are preparations containing two or more medicinal plants or their extracts in specific proportions. Their therapeutic concept is based on the possibility of complementary or synergistic actions of multiple phytoconstituents, which may provide broader pharmacological effects than individual herbs.[3] However, the therapeutic reliability of polyherbal formulations depends largely on their quality, consistency and standardization. Standardization involves establishing defined parameters for the identity, purity, strength, quality and consistency of herbal materials and finished products. Variations in plant species, geographical origin, cultivation, harvesting, processing, extraction and storage can significantly affect the chemical composition and biological activity of herbal formulations.

Quality control of polyherbal preparations is challenging because they contain numerous constituents originating from different plant sources. Important quality-control parameters include authentication of raw materials, physicochemical evaluation, phytochemical screening, detection of adulterants and contaminants, microbial testing and estimation of suitable marker compounds. Modern analytical techniques such as HPLC, HPTLC and chromatographic fingerprinting can assist in establishing chemical profiles and ensuring batch-to-batch consistency.[4,5] Therefore, systematic standardization is essential for improving the safety, efficacy, reproducibility and scientific acceptance of polyherbal formulations. The present review focuses on recent advances in the standardization and quality control of polyherbal formulations used for diabetes management, particularly authentication, phytochemical evaluation, marker-based analysis, chromatographic techniques and quality-assurance approaches.

2. Polyherbal Formulations in Diabetes Management

Polyherbal formulations are pharmaceuticals made from two or more plant species, based on the complementarity of their therapeutic properties. They have a background in traditional medical practices such as Ayurveda, Traditional Chinese treatment and Siddha treatment. The primary principles are increasing efficacy, reducing toxicity and thus fewer side effects. The active ingredients of PHFs are bioactive compounds such as alkaloids and flavonoids, saponins, tannins, and essential oils. PHFs are a significant research topic in modern phytotherapy because these substances can act through different physiological pathways and possess a broad spectrum of therapeutic effects.[6] Many phytochemicals are usually present in plants. Different plants or herbs mixed together can create a larger total medical impact because the constituent parts can work in concert with each other.[7]

Plant Name	Family	Parts Used	Active Principles	Mechanism of Action	Uses
Achyranthes aspera	Amaranthaceae	Leaves, seeds	Betaine, achyranthine	Affects carbohydrate metabolism	Hypoglycemic effect
Adhatoda vasica	Acanthaceae	Leaves	Vasicine, vasicinol	Supports glucose regulation	Antidiabetic
Allium cepa	Amaryllidaceae	Bulb	S-methyl cysteine sulfoxide	Stimulates pancreatic β-cells	Hypoglycemic effect
Allium sativum	Amaryllidaceae	Bulb	Allicin, alliin	Supports insulin secretion	Antidiabetic, antioxidant
Aloe barbadensis	Asphodelaceae	Leaves	Aloin, barbaloin, aloe-emodin	Supports glucose regulation	Hypoglycemic effect
Andrographis paniculata	Acanthaceae	Whole plant	Andrographolide	Supports insulin secretion	Antidiabetic, hepatoprotective
Areca catechu	Arecaceae	Leaves, flowers, seeds	Arecoline, arecaidine	Affects carbohydrate metabolism	Hypoglycemic
Azadirachta indica	Meliaceae	Leaves	Azadirachtin, nimbin, quercetin	Increase insulin signalling and glucose utilization	Antidiabetic, antioxidant
Bauhinia forficata	Fabaceae	Leaves	Kaempferitrin	Supports glucose metabolism	Hypoglycemic, antioxidant
Berberis aristata	Berberidaceae	Stem bark, roots, leaves	Berberine	Increase glucose transport and inhibits carbohydrate digestion	Hypoglycemic
Boerhavia diffusa	Nyctaginaceae	Leaves	Punarnavine, boeravinones	Supports glucose metabolism	Antidiabetic
Cassia auriculata	Fabaceae	Roots	Phytochemical compounds	α-Glucosidase inhibition	Antihyperglycemic
Citrullus indica	Cucurbitaceae	Aerial parts	Cucurbitacins, carotenoids	Affects insulin secretion and carbohydrate metabolism	Hypoglycemic
Commelina communis	Commelinaceae	Leaves, stem	Diterpenoid compounds	Inhibits α-glucosidase	Antihyperglycemic
Curcuma longa	Zingiberaceae	Rhizomes	Curcumin, turmerone	Inhibits α-glucosidase; affects GSK-3β	Antidiabetic, antioxidant
Cyperus rotundus	Cyperaceae	Whole plant	Cyperone, cyperene, cyperol	Inhibits intestinal glucose absorption	Hypoglycemic
Emblica officinalis	Euphorbiaceae	Fruits	Gallic acid, ellagic acid, vitamin C	Antioxidant action and reduced lipid peroxidation	Hypoglycemic, antioxidant
Enicostema littorale	Gentianaceae	Whole plant	Swertiamarin, sweroside, related compounds	Promotes glucose-induced insulin release	Hypoglycemic effect
Ficus benghalensis	Moraceae	Bark, leaves	Leucocyanidin, pelargonidin	Insulin secretion, glycogen synthesis	Antidiabetic
Ficus racemosa	Moraceae	Bark, leaves	β-sitosterol, racemosic acid, bergenin	Glycogenolysis and gluconeogenesis	Hypoglycemic activity
Mangifera indica	Anacardiaceae	Leaves	Mangiferin	α-Glucosidase-inhibiting activity	Hypoglycemic agent

Plant Name	Family	Parts Used	Active Principles	Mechanism of Action	Uses
Momordica charantia	Cucurbitaceae	Fruits	Momordin, momordicine, charantin	Insulin secretion, glycogen synthesis	Hypoglycemic agent
Pterocarpus marsupium	Leguminosae	Stem wood	Marsupsin, pterosupin, pterostilbene	Insulinomimetic activity	Antidiabetic
Syzygium aromaticum	Myrtaceae	Flower buds	Eugenol, caryophylline	Insulin secretion; carbohydrate digestion and absorption	Hypoglycemic agent
Syzygium cumini	Myrtaceae	Bark, seeds	Jambosine, jambolin, anthocyanins	α-Glucosidase-inhibiting activity	Antihyperglycemic
Terminalia arjuna	Combretaceae	Stem bark	Arjunic acid, arjunolic acid, gallic acid	Stimulates insulin release from islets	Hypoglycemic activity

List of Traditional plants used in management and treatment of Diabetes.[8]

3. Phytochemical Basis of Antidiabetic Activity

Many bioactive phytochemicals present in medicinal plants may exhibit antidiabetic effects through various biological processes. Main types of phytochemicals include flavonoids, phenolic compounds, alkaloids, terpenoids, saponins, tannins and polysaccharides. These substances may help to control blood glucose levels by affecting carbohydrate digestion, glucose absorption, insulin secretion and sensitivity, and glucose uptake. Moreover, many phytochemicals possess anti-inflammatory and anti-oxidant activities which could protect pancreatic β-cells from oxidative damage.[9-12]

• Major Phytochemical Classes

- **Flavonoids:** Flavonoids are polyphenolic compounds present in many medicinal plants. They may have antidiabetic effects by decreasing oxidative stress, improving insulin sensitivity and enhancing pancreatic β-cell function. Also, flavonoids were found to inhibit α-amylase and α-glucosidase enzymes involved in the digestion of carbohydrate.
- **Alkaloids:** Alkaloids are naturally occurring nitrogenous substances and can affect glucose

homeostasis in various ways. The possible effects of some alkaloids on insulin production, on glucose utilization and on carbohydrate metabolizing enzymes have been studied. However, these effects are not universal for all alkaloids, and require specific evidence for each molecule.

- **Terpenoids:** Terpenoids are natural substances with metabolic, anti-inflammatory and

antioxidant properties and can contribute to antidiabetic activity. Some terpenoids have been studied for their potential in improving insulin sensitivity and the regulation of glucose metabolism.

• Mechanisms of Antidiabetic Action

- **α-Amylase Inhibition** : One enzyme that can break down intricate carbohydrates into simpler sugars is called amylase. This enzyme can be inhibited by some plant polyphenols, which would reduce the breakdown of carbohydrates and lower the postprandial rise in blood glucose.[10,11]
- **AMPK Activation**: Amylase is one enzyme that breaks down complicated carbs into

simpler sugars. Some plant polyphenols may inhibit this enzyme and thus reduce the digestion of carbs and the increase in blood glucose after meals.

- **Improvement in Insulin Sensitivity:** Insulin resistant cells do not respond well to insulin. Certain phytochemicals may enhance insulin sensitivity through effects on glucose utilisation and insulin-signalling pathways. The magnitude of such an impact depends on the substance and the experimental or clinical data available.[10,12]

4. Phytochemical Standardization

Phytochemical standardisation is the process that helps to ensure quality and consistency of herbal and

polyherbal preparations by detection and quantification of some chemical constituents Chromatographic fingerprinting and marker analysis allow the evaluation of batch consistency and the estimation of variances originating from sourcing, processing and storage. [13,14]

• Phytomarkers

Phytomarkers are the specific chemical components or groups of components that are used to identify, describe or evaluate the quality of herbal products and preparations. The choice is based on chemical properties, analytical viability, availability of a reference standard, and applicability to the intended quality control purpose.

- **Active markers**: Active markers are constituents that have been shown to contribute to the therapeutic action of a herbal medicine. When the relation to the desired biological effect is established, their identification and quantification can help to assess the consistency of the formulations.[13,15]
- **Pharmacological markers**: Pharmacological markers are selected for a pharmacological

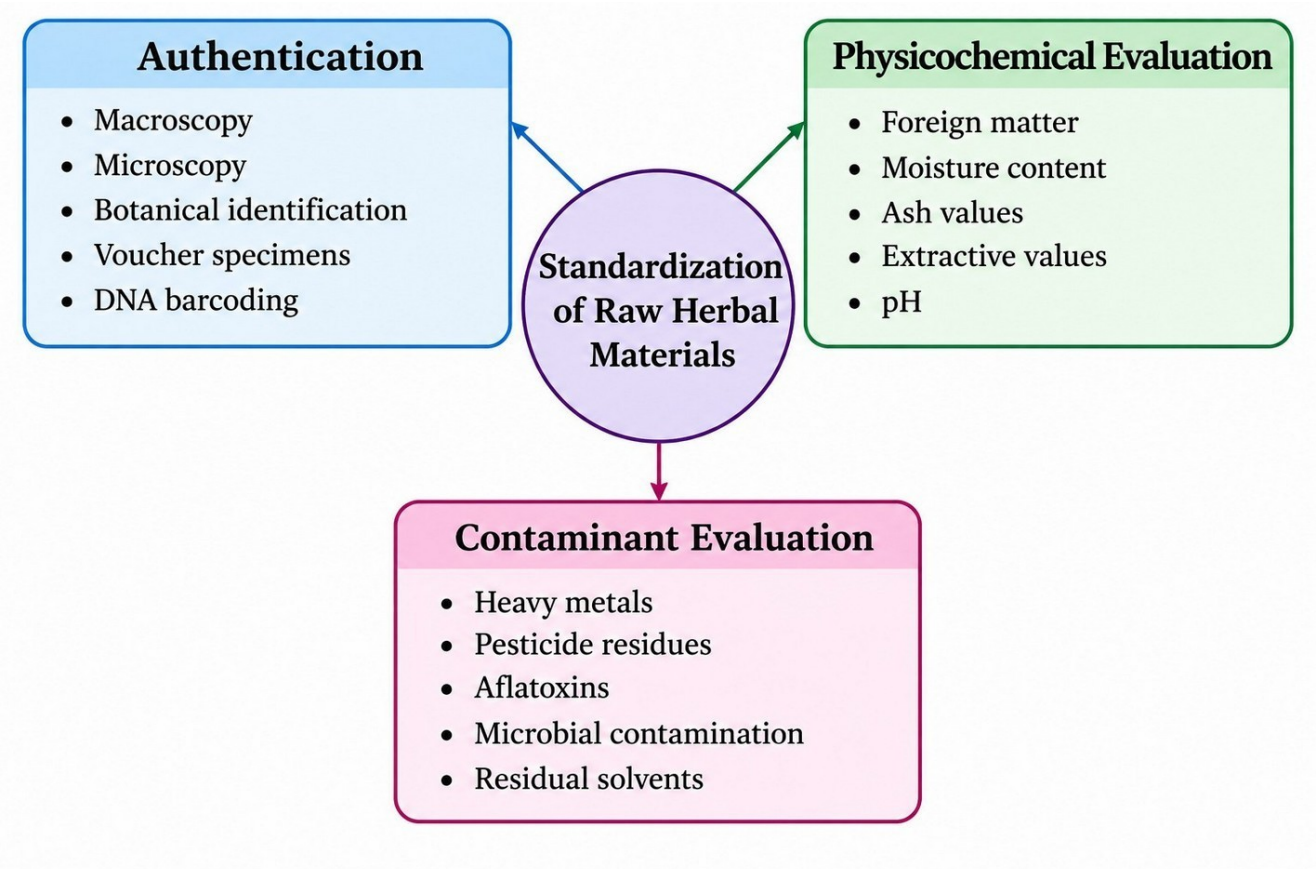
effect according to their known or studied association. Analytical measurements, backed up by suitable biological research, may improve quality assessment. However, it is necessary to establish rather than assume a marker's pharmacological significance based solely on its chemical presence.[15,16]

• Q-Markers

Quality markers or Q-markers are chemical components in herbal materials or formed during processing that are intended to represent the biological efficacy, safety, authenticity or quality of herbal therapeutic products. The idea is to make a link between the chemical composition and the product quality and, if there is data, the biological activity. This method may be particularly useful for the evaluation of complex formulations comprising multiple medicinal plants.[16]

- **Selection criteria**: When selecting Q-markers, consideration should be given to their suitability for routine quality evaluation, chemical specificity, traceability to the source material, availability of reliable analytical techniques and relevance to the expected therapeutic impact. Markers in relation to the contributions of individual constituents and the overall chemical profile should also be considered for polyherbal formulations.[15,16]
- **Stability**: Choosing Q-markers requires careful consideration of stability. For the marker to serve as a trustworthy indicator of product quality, it must be sufficiently consistent under specified manufacturing, storage, and analytical settings. If a component is unstable, it may be necessary to look into its degradation products and storage-related changes. [16,17]

5. Standardization of Raw Herbal Materials



Standardization is a process that ensures that each dose contains an established amount, quality, and therapeutic efficacy of ingredients. A herbal drug cannot be considered scientifically validated if the drug tested has not been authenticated and characterized to ensure consistency during the production process. Moreover, there are reports of various adverse effects and side effects of herbal drugs, including direct toxicity, hypersensitivity, contamination effects, and interaction with other drugs. The effectiveness of any herbal formulation is dependent on the phytochemical components it contains.[18]

• Authentication of Raw Herbal Materials

Authentication plays an important role in quality control of any raw herbal material. It ensures that a proper plant species has been used and prevents the use of a plant species substituted with another or contaminated with other materials. Macroscopic and microscopical examination is the first steps in the process of identification of an herbal material.

- **Macroscopy:** Macroscopy is the method of examining the material of the herbs by the naked eye or a low magnifying glass. The main qualities that are observed during the macroscopic examination are the form and size, color, odor, surface, texture, fracture, and the cut surface of the examined material. These qualities are compared to the described features or corresponding authentic specimens. Macroscopy is a simple and quick method that is mainly used for whole or cut medicinal plants.
- **Microscopy:** Microscopy is the examination of the internal structure and diagnostic features of plant material under a microscope. This technique is particularly useful for broken or ground material, where diagnostic characters are not visible to the naked eye. Stomata, trichomes, fibres, starch grains and calcium oxalate crystals may all be helpful in providing identification and indicating adulteration. Microscopy is often used in combination with other methods of identification.

- **Botanical Identification:** Botanical identification ensures a plant is correctly identified by its

scientific name with its distinctive features and taxonomy to ensure the proper use of a medicinal herb. This aids in preventing herbal substitution and makes it possible to acquire the right herbal material for the production of herbal medicine.[19]

• Contaminant Evaluation

Contaminant evaluation is an important step in the standardization of raw herbal materials. It ensures their safety, purity, and quality by detecting harmful substances.

- **Heavy Metals:** Herbal materials may get contaminated by heavy metals such as

lead, cadmium, mercury, and arsenic by soil and water. Their levels are tested to avoid toxicity.

- **Pesticide Residues:** These are traces of pesticides that stick to herbal medicines or lie in them. Testing is helpful to keep away the health risks that the substances may cause.
- **Microbial Contamination:** Bacteria, yeast, and moulds can be present in herbal materials due to harvesting, handling or storage. The presence of microbes is checked in the testing process. The test assures clients of the safety of the products. [20]

• Physicochemical Evaluation

- **Foreign organic matter:** This implies the removal of foreign substances in addition to the source plant in order to obtain the drug in pure form.
- **Ash values:** These serve assessment criteria for the identity and purity of crude drug, including total ash, sulphated ash, water soluble ash and acid insoluble ash, among others.
- **Moisture content:** Determining moisture content aids in reducing errors encountered in estimating the actual weight of drug material. Lower moisture levels indicate greater stability against product degradation.[21]

6. Analytical Standardization

• Conventional techniques

• UV-Visible spectroscopy Principal

Spectroscopy has unique spectra that are based on the principle of ultraviolet-visible radiation. Such spectra result from the chemical absorption of ultraviolet or visible light. Spectroscopy is based on the interaction between light and matter. After being absorbed, the material passes through several stages of excitation and re-excitation to form a spectrum. When an electromagnetic wave interacts with matter, it can undergo several processes: transmission, absorption, reflection, and dispersion. When radiation electronically modifies the structure of a molecule or ion, the object will show absorption in the visible or ultraviolet range. When the sample absorbs light in the ultraviolet or visible range, the sample's molecules are subjected to electronic alteration.[22]

• Applications

1. Drug analysis: determination of the contents of medicinal substances.
2. Drug evaluation: studying the action of new drugs.
3. Nucleic acid identification: determining DNA and RNA.[23]

• Thin Layer Chromatography Principal

Thin Layer Chromatography (TLC) works with the principle of differential adhesion. The components of a mixture differ in their affinity towards the stationary and mobile phases. Silica gel is a common stationary phase whereas an organic solvent or the mixture of solvents is the mobile phase. The components separate due to their different affinities towards the surface of the silica gel and their solubility in the mobile phase. Components with higher affinity towards the mobile phase travel faster and the ones with higher affinity towards the stationary phase move slower and hence get separated.[24]

• Chromatographic techniques

• HPLC

Principal

HPLC is used in pharmaceutical analysis for drug discovery, drug impurity analysis, and quality control. For food and beverage, it is used to detect contaminants and analyze nutritional components. It is used in environmental protection for the analysis of pollutants in water, air, and soil. In clinical chemistry, it is used for biomarker analysis, toxicology screening, and therapeutic drug monitoring. In forensic analysis, it is used to identify and quantify drugs and analyze trace evidence.

• Application

1. In toxicology drugs and toxicants are identified.
2. In pharmacology drugs are analyzed and controlled. [25]

• Gas chromatography Principal

Gas chromatography rests upon the principle that different components of a mixture will have different volatilities. The components are separated based on the volatility and the amount of time they spend interacting with the stationary phase. The most volatile component is the one which spends minimal time in the stationary phase and emerges out first while the least volatile component takes the longest time to emerge out from the column. All components have a characteristic retention time which is used to identify them as they emerge out of the Application

3. Food analysis.
4. Environmental monitoring.[26]

7. Chromatographic Fingerprinting

• HPTLC Fingerprinting

High-performance thin-layer chromatography (HPTLC) is widely used for the identification, authentication, and quality control of herbal materials. This method involves the separation of plant constituents on a coated plate and the analysis of resulting chromatographic patterns using UV light or specific detection reagents. Fingerprint profiles derived from HPTLC help compare the chemical similarity and diversity of herbal extracts. Itankar et al. (2015) conducted the work on the HPTLC fingerprinting of five antidiabetic herbs, including Gymnema sylvestre, Pterocarpus marsupium, Enicostema littorale, Syzygium cumini, and Emblica officinalis . The authors used phytochemical markers and retention factor (Rf) values to analyze the phytoconstituents of laboratory-prepared samples and commercial products. The results indicated that commercial and laboratory-made extracts had different chemical compositions, which demonstrated the importance of HPTLC fingerprinting in standardizing herbal preparations.[27]

• HPLC fingerprinting

High-performance liquid chromatography (HPLC) fingerprinting is a technique used to determine the identity, consistency, and quality of herbal medicines and polyherbal compounds. This method provides chromatographic profiles that allow the comparison of samples based on retention times and peak characteristics. Zhu et al. (2019) have developed an HPLC–DAD method for the determination of different samples of Chaenomelis Fructus. In the study, five characteristic peaks were identified and related to quinic acid, malic acid, protocatechuic acid, shikimic acid, and chlorogenic acid. The researchers have used similarity evaluation, principal component analysis (PCA), and hierarchical cluster analysis (HCA) to explore chemical differences between the samples and detect batches that may have been counterfeit. The method has demonstrated good precision, repeatability, and stability, which makes it a valuable tool for the authentication and assessment of herbal medicines. In addition, HPLC fingerprinting can be utilized in the analysis of polyherbal preparations to compare the differences in their chemical profiles.[28]

• Authentication

Authentication plays an important role in establishing the identity, purity and quality of medicinal plants used in herbal medicines. It helps in differentiating actual plant samples from adulterants, substitutes and misidentified species of plants. It is carried out by applying morphological, anatomical, chemical and molecular methods. Macroscopic and microscopic

examination are helpful in identifying plants based on their external and internal feature. On the other hand, chemical analysis helps identify the plants through their chemical constituents. In addition, DNA techniques such as DNA barcoding can be employed to identify plant species by analysing their DNA sequences. This will help in minimising adulteration, ensuring the authenticity of herbal raw materials and maintaining the quality of polyherbal formulations. [29]

• Detection of Adulteration

The detection of adulteration is an essential part of chromatographic fingerprinting, which aims to ensure the quality, safety, and authenticity of herbal medicines. Adulteration occurs when a mixture replaces a pure herbal drug, or when a herbal drug is mixed with undesirable substances or lower quality products. Chromatographic fingerprint analysis, including high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas chromatography (GC) helps detect different adulterants in herbal medicines. The HPLC method provides the retention time, peaks, and percentage of each component in the mixture. It helps compare the peaks of the pure drug sample and adulterated sample. Spectroscopy involves near-infrared spectroscopy and uses a non-destructive approach to analyze herbal medicines. It is also possible to use molecular techniques such as DNA barcoding, for determining mixed species and ensuring the quality of herbal medicines. Chemometric tools can also be applied in the separation and classification of mixed samples, and identification of pure samples from adulterated samples. All these methods can be used together to determine adulteration and improve the quality of herbal medicines.[30]

8. Metabolomics-Based Standardization

• Targeted metabolomics

Herbal medicine research makes use of metabolomics to investigate the chemical composition of medicinal plants and herbal formulations. Metabolomics contributes to the identification of bioactive compounds and facilitates comprehension of interindividual differences in the chemical profiles of herbal preparations. Liquid chromatography–mass spectrometry (LC-MS) presents high sensitivity and wide metabolite coverage, enabling the detection of low-abundance compounds. This technique is relevant to the analysis of complex herbal extracts and to the identification of phytochemicals such as flavonoids, alkaloids and glycosides. Capillary electrophoresis–mass spectrometry (CE-MS) is an emerging approach to targeted profiling of alkaloids and phenolic compounds. Metabolomic profiling combined with chemometric approaches can contribute to the authentication of herbal formulations, and can assist in the evaluation of batch-to-batch consistency in addition to distinguishing between genuine formulations and adulterated or substandard products.[31]

• Untargeted metabolomics

Untargeted metabolomics opens the possibility for an in-depth exploration of all metabolites present in medicinal plants and herbal preparations. Liquid chromatography – mass spectrometry allows the detection of a large range of different molecules like amino acids, sugars, organic acids, lipids, flavonoids, and phenolics. Unlike targeted metabolomics, this study evaluates a wide array of all kinds of metabolites detectable by mass spectrometry, and not limited to a specific set of compounds. Using statistical analysis, such as principal component analysis, it becomes possible to evaluate the relationships between the detected variables. Therefore, it can be concluded that untargeted metabolomics can help assess chemical variability and identify markers of quality in herbal and polyherbal drugs.[32]

• Metabolic fingerprinting

Metabolic fingerprinting is an analytical strategy used to determine the overall chemical composition of herbal materials and polyherbal formulations. It facilitates the identification and comparison of phytochemical constituents and contributes to the evaluation of the quality and consistency of herbal products. Dinakaran et al. examined marketed antidiabetic polyherbal formulations by means of spectroscopic techniques, including UV and IR, in addition to chromatographic techniques, including TLC and HPTLC. The investigation revealed the presence of multiple phenolic constituents and demonstrated that HPTLC fingerprinting could be applied to the quality control of polyherbal formulations.[33] Moreover, metabolic fingerprinting

based on MS and NMR presents a more comprehensive approach to the quality control of herbal materials and phytopharmaceuticals. Such techniques assist in characterizing complex chemical profiles and contribute to the evaluation and standardization of herbal products. [34]

9. Chemometrics in Polyherbal Standardization

• PCA and HCA:

Principal component analysis (PCA) identifies the major patterns of variation in chromatographic or spectral fingerprints, and hierarchical cluster analysis (HCA) groups herbal samples in clusters according to their chemical similarity. These approaches can be useful in herbal authentication and in the control of the consistency of batches of a given herb. [35]

• PLS-DA

Partial least squares–discriminant analysis (PLS-DA) uses predefined groups of samples to build up a model that allows the discrimination of samples. When properly validated, this model can classify authentic and adulterated samples.[36]

• Applications:

The combination of HPLC, HPTLC, GC–MS or FTIR data with appropriate multivariate models enable the authentication, screening for possible adulterants, and classification of herbal medicines.[35,36]

10. Biological Standardization

Biological standardization is carried out through in-vitro assays, cellular models, and in-vivo experimental models to determine the biological activity, efficacy, and safety of polyherbal formulations. In diabetes research, the glucose-lowering activity, inhibition of enzymes involved in carbohydrate digestion and glucose uptake, anti-inflammatory effects, and the possible protective effects of herbal formulations on pancreatic β-cells can be evaluated using these models. The choice of these models depends on the targeted mechanism of action and the composition of the formulation**.**

• In-Vitro Assays

In-vitro assays are laboratory tests that are performed outside a living organism in order to study the biological activities of herbal extracts and polyherbal formulations. Such assays are useful for preliminary screening, for comparing formulations and for identifying potential mechanisms of antidiabetic activity.

- **α-Amylase inhibition:** During digestion, α-amylase degrades complex carbohydrates into smaller sugars. Inhibiting this enzyme may decelerate carbohydrate digestion and thus help attenuate the rapid elevation of postprandial blood glucose levels. The inhibitory activity of polyherbal formulations can be measured by testing the enzyme activity in both the presence and the absence of the test extract. However, positive results in this assay do not necessarily demonstrate clinical antidiabetic efficacy.
- **α-Glucosidase inhibition:** α-Glucosidase participates in the final phases of carbohydrate

digestion, transforming oligosaccharides and disaccharides into absorbable monosaccharides. In-vitro inhibition assays are employed to determine whether herbal extracts can reduce this enzyme activity and potentially delay glucose absorption. These assays can help compare the inhibitory potential of various formulations and contribute to the investigation of their possible effects on postprandial hyperglycaemia. [37,38]

- **Glucose uptake:** Glucose uptake assays measure the capacity of cells to take up glucose

from the surrounding medium. Common experimental systems include skeletal muscle cells, adipocytes and other appropriate cell models. Increased glucose uptake may indicate improved cellular glucose utilization and may be associated with insulin-related signalling or the regulation of glucose transporters, including GLUT4 translocation in relevant cell types. Determining the mechanism involved in such findings may need further investigation.

- **Anti-inflammatory assays**: Chronic low-grade inflammation is related to insulin

resistance and the development of type 2 diabetes. Anti-inflammatory activity can be examined by using appropriate cell-based assays that measure inflammatory mediators such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and other markers. These assays assist in determining whether a polyherbal formulation can modulate inflammatory responses in the selected experimental conditions.

• Cellular Models

Cellular models provide a means of examining how polyherbal formulations affect particular cell types participating in the regulation of glucose and in insulin secretion. Before further assessment in animals, they provide information about cellular responses, possible mechanisms of action and potential toxicity.

- **Pancreatic β-cell Models:** Pancreatic β-cells are the cells that produce and secrete insulin when blood glucose levels rise. INS-1 and MIN6 are among the cell lines commonly used to examine insulin secretion, glucose responsiveness and β-cell function. Polyherbal extracts can be tested for their ability to protect β-cells from experimentally induced damage, to reduce oxidative stress and to maintain insulin-secretory function. Assays of cell viability and insulin secretion can help evaluate these effects. However, protective activity observed in cultured cells does not necessarily mean that the same effect occurs in living organisms. [39,40]

• In-vivo Models

In-vivo models involve the evaluation of polyherbal formulations in living experimental animals. They provide information about the regulation of blood glucose, insulin-related responses, metabolic changes and possible toxic effects. Since different models replicate different features of diabetes, the model should be chosen based on the goal of the study.

- **Streptozotocin (STZ)-induced Diabetes:** Streptozotocin is a diabetogenic compound that

harms insulin-producing pancreatic β-cells. It is widely used to induce experimental diabetes in rodents. This model is useful for assessing alterations in blood glucose, insulin levels and pancreatic histology, as well as the effects of antidiabetic treatments. Based on the dose and experimental protocol, STZ may cause significant β-cell damage or be combined with dietary interventions to examine features similar to type 2 diabetes.

- **Alloxan-induced Diabetes:** Alloxan is a further chemical agent used to induce

experimental diabetes through pancreatic β-cell toxicity that involves oxidative damage. This model is used by researchers to assess the effects of herbal formulations on blood glucose, insulin secretion and changes in pancreatic tissue. However, its effectiveness may vary depending on animal species, dose and experimental conditions, and acute hypoglycaemia as well as other toxic effects are significant limitations.[41,42]

- **High-fat-diet (HFD) models:** High-fat-diet models are used to examine obesity-associated insulin resistance and metabolic disturbances related to type 2 diabetes. Animals fed a high-fat diet may develop increased body weight, decreased insulin sensitivity and glucose regulation problems. Combining a high-fat diet with low-dose STZ may further impair β-cell function and lead to a wider range of phenotypic features similar to type 2 diabetes. These models are useful for assessing whether polyherbal formulations improve glucose tolerance, insulin sensitivity and connected metabolic abnormalities. However, the results depend on diet composition, treatment duration, animal strain and experimental protocol.[42,43]

11. Chemical–Biological Correlation

• Phytochemical profile:

The chemical profile of herbal extracts can be determined by using HPTLC, HPLC and LC-MS for generating fingerprints of polyherbal preparations.[44]

• Marker identification:

The choice of chemical markers should be dictated by their analytical properties and potential relevance to the mechanism of action, which requires experimental validation. [13,44]

• Biological activity:

The biological activity of a preparation can be accessed though assays for α-amylase inhibition, α-glucosidase inhibition and glucose-uptake. [45]

• Correlation analysis:

By using statistical and chemometric approaches, the biological activity data can be related to the chemical profile information for identifying bioactive constituents. The putative bioactive compounds need to undergo further investigation to establish structure-activity relationships. [13,44]

12. Adulteration and Authentication

• Plant Substitution

Plant substitution, in which the intended therapeutic plant or plant portion is substituted with another species or an unsuitable plant material, is a significant quality-control issue in herbal medicines. Local names, morphological similarities, or challenges distinguishing between powdered and dried materials can all lead to unintentional substitution. It may also be done on purpose to make money by substituting less expensive therapeutic herbs for more costly ones. Such replacements may change the phytochemical makeup of herbal preparations and lessen their anticipated medicinal efficacy. For instance, there have been reports of using related species in herbal medicines and substituting the aerial parts of Withania somnifera for its roots. In order to detect plant replacement and guaranty the quality of herbal goods, botanical identification, comparison with verified reference materials, DNA-based techniques, and chemical fingerprinting are crucial. [45, 46]

• Incorrect Botanical Identification

Similarities between closely related plant species' microscopic and morphological traits might lead to incorrect botanical identification. When medicinal plants are dried, powdered, or made into extracts, their distinctive characteristics may be lost, making identification especially challenging. The therapeutic activity, safety, and consistency of a product can all be impacted by the usage of raw components that are not appropriately identified. Voucher specimens, taxonomic verification, macroscopic and microscopic analysis, and DNA barcoding can all be used to identify medicinal plants. However, because DNA can be broken down or present in insufficient amounts in highly processed extracts, DNA-based techniques might be less successful. Thus, the accuracy of plant identification can be increased by combining botanical examination with chemical analysis and DNA-based verification. [45,47]

• Synthetic Drug Adulteration

The unreported addition of pharmaceutical or synthetic ingredients to herbal goods is known as synthetic medication adulteration. Such adulteration may be done to deceive customers, boost business earnings, or create quick or exaggerated medicinal benefits. When customers believe a product is made entirely of natural materials, undeclared pharmaceutical substances can lead to major safety concerns, drug interactions, and unanticipated negative effects. Suspected synthetic adulterants can be identified and quantified with the use of analytical methods such gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), and high-performance liquid chromatography (HPLC). The chemical characteristics of the suspected substance and the makeup of the herbal product determine which approach is best. Reliable detection requires validated analytical methods and suitable reference standards. [46,48]

• Contamination

Herbal medicines may become contaminated while being grown, harvested, dried, processed, transported, and stored. Heavy metals, pesticide residues, bacteria, mycotoxins, and leftover solvents are significant pollutants. Polluted soil or water, poor hygiene, improper storage conditions, or subpar production techniques can all lead to contamination. Metals, microbiological contaminants, and mycotoxins were found to be the most commonly reported contamination categories in a comprehensive assessment of herbal therapeutic items from low- and middle-income countries. These impurities could jeopardize product safety and expose customers to hazardous microbes or poisonous compounds. Therefore, in accordance with the product and relevant standards, quality-control programs should incorporate suitable testing for heavy metals,

microbiological limits, pesticide residues, mycotoxins, and residual solvents [49,50].

• HPLC Authentication

The chemical components of herbal remedies are frequently analyzed both qualitatively and quantitatively using high-performance liquid chromatography (HPLC). It creates a chromatographic profile that can be compared with a verified reference sample by separating substances based on how they interact with the stationary and mobile phases. When appropriate analytical conditions and reference standards are available, HPLC can be used to quantify specific chemical markers, analyze batch differences, and identify some known adulterants. Chemical constituent identification and characterisation can be enhanced by combining HPLC with mass spectrometry or a diode-array detector. However, the full botanical identity of a complex herbal mixture might not be established by the identification of just one or a few marker chemicals. Stronger proof of product quality and authenticity can be obtained by combining chromatographic fingerprinting with complementary authentication methods and multi-marker analysis. [46,48]

• AI-Assisted Authentication

Emerging methods for authenticating and evaluating the quality of medicinal plants include artificial intelligence (AI) and machine learning. These methods can discover patterns linked to various plant species or sample-quality categories by analyzing digital photos, chromatographic fingerprints, spectroscopic data, and metabolomic profiles. Complex chemical datasets can be classified and compared using chemometric techniques such as principal component analysis (PCA), hierarchical cluster analysis (HCA), and partial least-squares discriminant analysis (PLS-DA). Machine-learning models can be used in conjunction with validated analytical measures to evaluate batch-to-batch variance, identify odd samples, and detect potential adulteration. However, the quality of the reference samples, suitable model validation, and representative training datasets all affect how reliable they are. Therefore, traditional botanical identification and laboratory-based authentication techniques should be complemented rather than replaced by AI-assisted methodologies. [46, 47]

13. Regulatory Perspectives

• World Health Organization (WHO):

WHO provides guidelines on quality control of herbal materials, including botanical identification, purity, foreign matters, pollutants, and analytical testing.[51]

• Ayurveda, Siddha, Unani and Homoeopathy (AYUSH):

By way of applicable legislation, quality requirements, and pharmacopoeial standards, the Ministry of AYUSH, Government of India, supports the regulation and standardization of traditional medicines.[52]

• Indian Pharmacopoeia (IP):

Published by the Indian Pharmacopoeia Commission, the IP sets out official quality standards for drugs listed in its monographs, which include requirements for identity, purity, strength, and testing.[53]12 Nov 2026

• Ayurvedic Pharmacopoeia of India (API):

The API sets down standards for Ayurvedic medicinal plant materials and ingredients, thereby supporting

botanical authentication, purity assessment, and quality control.[54]

• United States Pharmacopeia (USP):

USP develops quality standards and analytical methods for botanical ingredients and dietary supplements, supporting identity verification, contaminant testing, and product consistency.[55]

• European Pharmacopoeia (Ph. Eur.):

The European Pharmacopoeia provides monographs and analytical requirements for herbal drugs and preparations, covering identification, purity, and assay testing.[56]

• Food and Drug Administration (FDA):

In its Botanical Drug Development Guidance for Industry, the FDA draws attention to the quality-related difficulties associated with botanical products, as these products are comprised of complex mixtures rather than one active ingredient that is chemically defined. It gives emphasis to characterization of raw materials, manufacturing controls, chemical testing, and evidence that supports safety and efficacy.[57]

KEY FINDINGS OF THIS REVIEW

This review identifies an integrated, multi-parameter approach as the most informative strategy for assessing the quality of polyherbal formulations studied for diabetes management. The article's central findings are that botanical authentication establishes the identity of raw materials; physicochemical and contaminant testing assess purity and selected safety risks; marker-based analysis and chromatographic fingerprints help compare chemical composition across batches; and metabolomics with chemometric tools can provide broader profiles and reveal sample variation. Biological assays can explore possible antidiabetic activity, but laboratory or animal findings do not independently prove clinical effectiveness. The review therefore supports combining validated analytical methods with consistent manufacturing, suitable safety assessment and well-designed clinical studies.

COMPARATIVE VIEW OF QUALITY-CONTROL APPROACHES

Approach	Main contribution	Important limitation
Single-marker analysis	Relatively simple routine monitoring of a selected constituent.	One marker may not represent the full complexity of a polyherbal mixture.
Multi-marker / fingerprint analysis	Broader chemical characterization and batch comparison.	Needs validated methods, reference materials and interpretation.
Metabolomics + chemometrics	Can reveal wider patterns and sample differences in complex mixtures.	Requires suitable instrumentation, expertise and robust validation.
Biological assays	Investigates possible mechanisms and biological activity.	Preclinical activity alone does not establish clinical efficacy.

Note on percentages:

The supplied review does not present a single comparable dataset from which valid percentages or a numerical comparison chart can be calculated. No percentages have therefore been invented. The schematic and comparison table represent qualitative findings from the article.

PICTORIAL / GRAPHICAL REPRESENTATION

2. Test purity & safety

Physicochemical tests; contaminant screening

3. Profile chemistry

Phytochemical markers; TLC/HPTLC/HPLC/GC

1. Authenticate raw plants

Botanical, macro/microscopy, DNA methods

←

5. Evaluate activity

In-vitro/cellular/in-vivo and clinical evidence

4. Compare profiles

Fingerprinting, metabolomics and chemometrics

6. Assure quality

Validate methods; control manufacturing and batches

Integrated quality-control workflow for polyherbal formulations studied in diabetes management.

CONCLUSION

Standardization and quality control are essential for improving the reliability of polyherbal formulations investigated for diabetes management. Botanical authentication, physicochemical testing, contaminant screening, phytochemical and marker analysis, and chromatographic fingerprinting provide complementary information about identity, purity and consistency. Emerging approaches such as metabolomics and chemometric etric analysis can broaden chemical characterization and help detect batch variation or adulteration. Biological assays can add evidence about possible activity, but they should be interpreted alongside chemical data and should not be treated as proof of clinical benefit. Future progress depends on validated analytical methods, reproducible manufacturing practices, appropriate safety evaluation and well-designed clinical studies. Polyherbal products should not replace prescribed diabetes treatment without advice from a qualified healthcare professional.

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