

# Pharmacognostic Standardization and Quality Controlled Parameters of Polyherbal Formulations

S. Shruti<sup>1\*</sup>, Pooja Sharma<sup>2</sup>, Pradeep Dhaked<sup>1</sup>, Rishu Sharma<sup>3</sup>, Aaroohi Parmar<sup>3</sup>

<sup>1</sup>Department of Rasashastra evam Bhaishajyakalpana, Sri Ganganagar College of Ayurvedic Science and Hospital, Sri Ganganagar, Rajasthan 335002.

<sup>2</sup>Department of Agad Tantra evam Vidhi Vaidyaka, Sri Ganganagar College of Ayurvedic Science and Hospital, Sri Ganganagar, Rajasthan 335002.

<sup>3</sup>Department of Shalakyta Tantra, Sri Ganganagar College of Ayurvedic Science and Hospital, Sri Ganganagar, Rajasthan 335002

\*Corresponding Author E-mail: [drshrutiswami@gmail.com](mailto:drshrutiswami@gmail.com)

## ABSTRACT

This is a synthesis review of animal evidence on the pharmacognostic standardization and quality-controlled parameters of polyherbal preparations extending to macroscopic and microscopic validation, physicochemical and phytochemical profiling, chromatographic fingerprinting (HPTLC/HPLC), and in vivo pharmacological validation in rodents. Revolving around preclinical research, the review records that morphologically identical, ash and extractive value and chemical fingerprint standardized formulations are more consistent in and reproducible therapeutic effects, mostly which are anti-inflammatory, hepatoprotective, antioxidant, and antidiabetic, in Wistar, Sprague Dawley and Swiss albino. Associations of the integrity of chromatographic markers with normalization of biochemical indices (ALT, AST, ALP, SOD, CAT, GPx and fasting glucose) highlight the practical relevance of analytical-biological analysis. Nonetheless, there are still unresolved inconsistencies such as inter-laboratory inconsistency, lack of harmonized world standards regarding multi-herbal products, lack of application of sophisticated analytics (LC-MS, NMR, metabolomics), and insufficient research on herb-herb pharmacokinetics/pharmacodynamics. Standardized pharmacognostic-analytical protocols, integration of contemporary chemometric and omics approaches, development of validated digital reference libraries and greater long-term and mechanism-based animal studies should therefore be adopted to enhance quality assurance and translational potential of polyherbal therapeutics.

## Key Words:

Pharmacognostic Standardization; Polyherbal Formulations; Quality Control; Chromatographic Fingerprinting; Physicochemical Parameters; Animal Models; Hepatoprotective; Anti-Inflammatory

## Article History:

Received Aug 01, 2025

Revised Sep 20, 2025

Accepted Oct. 18, 2025

Published Oct. 28, 2025

DOI: <https://doi.org/10.64063/3049-1681.vol.2.issue10.2>

## 1. INTRODUCTION

The growing acceptance of herbal and traditional medicines across the world has generated a resurgence in the creation of scientifically proven and controlled formulations<sup>1</sup>. Polyherbal formulations, i.e. a mixture of more than two medicinal plants with the aim to boost

**International Journal of Pharmacognosy and Herbal Drug Technology (IJPHDT)**

ISSN: 3049-1630 | Vol. 02 Issue 10, Oct.-2025 | pp. 08-22

Int. J. Pharmacogn. Herb. Drug Technol.

<https://www.aktpublication.com/index.php/ijphdt>

pharmacological efficacy, are especially conspicuous considering their synergistic pharmacological effects and fewer side effects than the case of single-plant preparations. Nevertheless, even though such formulations have potential in therapy, their reliability and reproducibility are largely determined by standardization. The insensitivity of bioactive profiles due to the variation in the source of plant materials, environmental factors, and the methods used can cause serious safety and efficacy problems. It is therefore necessary to put in mind a comprehensive pharmacognostic and quality management frame to warrant the uniformity, identity, purity and potency of polyherbal formulations before they could be used in experimental and therapeutic conditions.



**Figure 1:** Polyherbal Formulations<sup>2</sup>

Over the recent years, animal research has proved to be essential in validation of the pharmacognostic and pharmacological features of polyherbal preparations. These clinical trials can be used to derive good insights into the biological applicability of structured herbal products that will serve as a linkage between traditional medicine and evidence-based pharmacotherapy. The parameters that can be evaluated systematically in the experimental models on rats and mice include anti-inflammatory, hepatoprotective, antioxidant, and antidiabetic activity under controlled laboratory conditions. Moreover, pharmacognostic standardization along with animal testing can be used to determine the therapeutic efficacy in a way that it determines possible toxicity and dosage safety margins. This two-fold strategy of integrating botanical characterization and validation *in vivo* is, therefore, one of the scientific avenues of credibility and regulatory approval of polyherbal preparations on the contemporary pharmacology.

### **1.1. Background and Context**

Polyherbal preparations, mixtures of several plant-based substances have been the foundation of traditional medical systems including Ayurveda, Siddha and Unani. Such combinations are explained by the need to have synergistic therapeutic effects, broad spectrum activity, and optimization of safety. Randomness in the quality of raw materials, extraction procedures, and phytochemical make-up however, presents an issue of reproducibility and clinical reliability. Thus, standardization of pharmacognostics, including morphological, histological,

physicochemical, and phytochemical analysis is the basis of the guarantee of constant efficacy and safety.

### 1.2.Objectives of the Review

This review seeks to:

- To evaluate pharmacognostic and physicochemical parameters of polyherbal formulations.
- To analyze chromatographic fingerprinting methods for chemical standardization.
- To assess animal-based studies validating therapeutic efficacy and safety.
- To identify strengths, limitations, and gaps in current standardization practices.
- To propose an integrated framework for quality control and preclinical validation.

### 1.3.Importance of the Topic

In the present world scenario where there is increased use of herbal therapeutics, it is critical that quality and reproducibility of formulations be ensured. Animal studies provide a biologically realistic and ethically controllable system of drug validation and toxicity testing that connects the gap between in vitro studies and clinical trials. Standardisation does not only serve to validate the traditional medicinal knowledge using scientific rigor but also preconditions the creation of globally accepted herbal products that are safe, effective and pharmaceutically consistent<sup>3</sup>.

## 2. PHARMACOGNOSTIC EVALUATION FRAMEWORK AND PRECLINICAL RESEARCH FINDINGS

As mentioned in this section, the standardization of polyherbal formulations depends on pharmacognostic, physicochemical and chromatographic analyses which are then validated using animal models to ascertain their effectiveness and safety. Nevertheless, they cannot be reproducible or clinically applicable due to the absence of international standardization, inter-laboratory consistency, and sophisticated integration of their analysis.

### 2.1.Summary of Key Research Studies

Many of the preclinical and pharmacognostic studies have been conducted on assessing the standardization and quality control parameters of polyherbal formulations, especially those applied in the traditional medicine systems like Ayurveda and Siddha. As an example, polyherbal anti-inflammatory-based formulations, which included *Withania somnifera* (Ashwagandha), *Curcuma longa* (Turmeric), and *Boswellia serrata* (Shallaki) have been standardized in a systematic way through physicochemical parameters such as the loss of dry matter, the total ash, the acid-insoluble ash, and the alcohol and water-soluble extractive values. Biological testing was then performed on these standardized formulations using the Wistar models of rat subjects in carrageenan induced paw edema experiments which showed a significant amount of anti-inflammatory activity that was comparable to the standard drugs like diclofenac sodium<sup>4</sup>.

Equally, Phytochemicals Phytoprotective preparations using *Phyllanthus niruri*, *Boerhavia diffusa* and *Andrographis paniculata* have demonstrated impressive potentials in alleviating hepatotoxicity in paracetamol-induced rat models. These researches noted significant change in the serum biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin with restoration of normal histopathological appearances of hepatic tissues. Such animal-based studies have been included to affirm the efficacy of the therapeutic efficacy and safety of polyherbal preparations after combining pharmacognostic validation with pharmacological evidence.

## 2.2.Methodologies and Findings

Polyherbal formulations are usually standardized and evaluated through a multi-step approach that includes morphological, physicochemical, phytochemical, chromatographic as well as pharmacological testing:

- **Macroscopic and Microscopic Analysis:** Morphological and anatomical examination is carried out to ensure that every herbal ingredient is pure and right. Microscopic methods are used to observe their characteristic features, e.g. trichome structure, xylem and phloem structures, calcium oxalate crystals, starch grains and secretory canals. The diagnostic markers are used to identify adulteration or raw materials replacement.
- **Physicochemical Parameters:** The identification of parameters such as the pH, total ash, insoluble ash in sulphuric acid, insoluble ash in alcohol, moisture content and extractive values (in water and alcohol) are used to provide consistency and quality between batches. These values play a crucial role as they are critical signs of purity, stability and concentration of active constituents in formulations<sup>5</sup>.
- **Phytochemical Screening:** Pre-qualitative assays are conducted to determine the major classes of bioactive elements, such as alkaloids, flavonoids, tannins, glycosides, phenols, terpenoids, and saponins. The availability of these compounds gives an opportunity to correlate the observed pharmacological effects with certain phytoconstituents.
- **Chromatographic Profiling (HPTLC/HPLC):** High-Performance Thin Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) are some of the advanced chromatographic methods used to produce unique chemical fingerprints with the different formulations. These profiles serve as authentication identify and are used in ensuring the integrity and traceability of herbal formulations.
- **Animal Pharmacological Models:** Pharmacological tests in vivo are performed with the standardized animal models. Rat or mouse models of anti-inflammatory, antioxidant, hepatoprotective, antidiabetic and analgesic are evaluated. Measures on parameters like edema inhibition, blood glucose levels, serum enzyme profiles and tissue histopathology are taken to support the purported therapeutic efficacy.

## 2.3.Critical Evaluation of Strengths and Weaknesses

**Strengths:**

- The utilization of animal models offers great biological validation, as it allows a comprehensive insight into toxicity, therapeutic potential and dose-response relationships, under controlled experimental settings.
- Coupling the chromatographic, pharmacognostic and histological data increases the reproducibility and validity of the research results.
- Multiple parameters such as physicochemical and phytochemical profiles are subjected to cross-validation to guarantee authenticity, quality assurance and standardization of polyherbal formulations.
- Such multidisciplinary strategies fill the gap between the traditional knowledge and the modern scientific validation, enhancing the popularity of the herbal formulation around the world<sup>6</sup>.

**Weaknesses:**

- The inter-laboratory protocols are not standardized which causes inconsistency of results between various studies and laboratories.
- Most of the literature does not consider pharmacokinetic and pharmacodynamic interactions between the constituent herbs that can affect the therapeutic outcome or trigger unintended adverse effects.
- Lack of globally recognized quality standards and regulatory guidelines of multi-herbal formulations negatively affects global commercialization and clinical translation.
- The application of modern analytical methods, including LC-MS, NMR, and metabolomics, is minimally applied which limits the complete characterization of active constituents and bioavailability profiles.

Altogether, although animal-based pharmacognostic tests present a solid baseline of the therapeutic and safety characteristics of polyherbal preparations, global harmonization, sophisticated analytical assimilation and clinical authentication are urgently required to provide uniform effectiveness, reproducibility, and consumer trust.

**Table 1:** Table 1: Review of Studies on Polyherbal Formulation Standardization<sup>7</sup>

Author(s) & Year	Study Title	Focus Area	Methodology	Key Findings
Dinakaran et al. (2018) <sup>8</sup>	Profiling and determination of phenolic compounds in Indian marketed hepatoprotective polyherbal formulations	Identification and quantification of phenolic compounds in hepatoprotective formulations	Advanced chromatographic techniques used for profiling bioactive phenolic constituents	Revealed significant variation in phytochemical content and antioxidant potential, highlighting the need for formulation

				standardization and quality control
<b>Gupta et al. (2022)<sup>9</sup></b>	Development of a gastroretentive polyherbal formulation and its standardization	Enhancement of bioavailability and therapeutic efficacy through formulation development	Formulation optimization using pharmacognostic and physicochemical standardization parameters	Demonstrated that gastroretentive and controlled-release systems improve pharmacokinetic profiles and provide a scientific basis for advanced herbal delivery systems
<b>Gupta et al. (2021)<sup>10</sup></b>	Review on quality control parameters for standardization of herbal drugs	Standardization and quality control of herbal formulations	Review of pharmacognostic, physicochemical, and chromatographic evaluation techniques	Highlighted the need for integrating modern analytical tools with traditional methods to ensure uniformity, purity, and potency in herbal drugs
<b>Haque et al. (2020)<sup>11</sup></b>	Pharmacognostical, physicochemical, and contamination studies of polyherbal formulation “Qurse Pudina”	Evaluation of safety, authenticity, and quality of a marketed polyherbal formulation	Morphological, microscopic, physicochemical, microbial, and heavy metal analyses	Confirmed safety and authenticity; emphasized importance of contamination control and standardization for consumer safety
<b>Harinarayanan et al. (2024)<sup>12</sup></b>	Pharmacognostic characterization of Panchatikta, an Ayurvedic polyherbal combination	Validation and standardization of traditional Ayurvedic formulation	Macroscopic, microscopic, and chromatographic analyses for phytochemical consistency	Validated traditional Ayurvedic formulation through pharmacognostic techniques and highlighted modern tools for ensuring herbal product reliability

### 3. STANDARDIZATION PARAMETERS AND EXPERIMENTAL VALIDATION OF POLYHERBAL FORMULATIONS

Pharmacognostic, physicochemical, chromatographic, and animal-based studies provide a combination of ensuring authenticity, consistency, and effective action of polyherbs in knowledge. These combined standardization technologies scientifically authenticate conventional herbal medicine because they relate morphological, chemical and biological parameters to treatment effects<sup>13</sup>.

#### 3.1. Pharmacognostic Evaluation

Pharmacognostic standardization is the basic procedure in the validation of the identity of purity and quality of polyherbal formulations. This is done mainly by thorough morphological and anatomical examination of each plant material that is incorporated in the formulation to validate it. As an example, polyherbal preparations of *Ocimum sanctum* (Tulsi) and *Tinospora cordifolia*

(Guduchi) tested on animals were carefully analyzed regarding characteristic features of diagnosis such as glandular and non-glandular trichomes, parenchymatous tissues and lignified xylem fibres. These structural features are unique identifiers of botanical authentication, which is prescribed to assuring that nothing but pure and authentic plant materials are utilized in additional pharmacological testing.

Microscopic validation will, therefore, ensure the removal of adulterated and substitutions that may affect the pharmacological effects. Rat-based testing showed that the formulations with standardization with the help of pharmacognostic examination had a birth order of consistency in therapeutic effects, which confirmed the real character of the pharmacological operations as the actual constituents of the plant, as opposed to external impurities or replacements. Those standardizations not only enhance consistency of experimental findings, but in addition enable a scientific basis of morphological identity-biological efficacy correlation<sup>14</sup>.

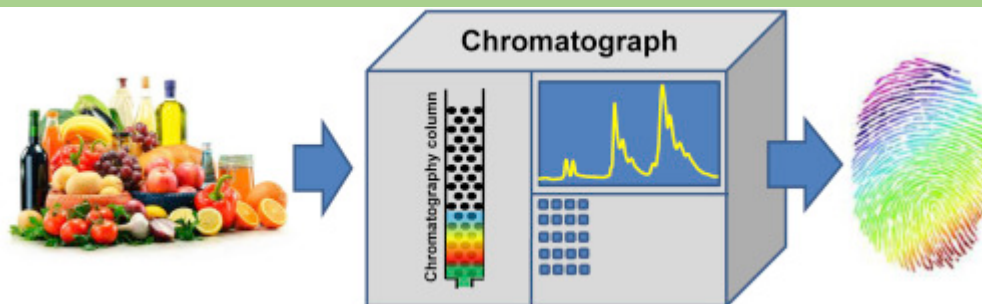
### 3.2. Physicochemical and Phytochemical Parameters

The physicochemical and phytochemical analysis of polyherbal preparations guarantee the chemical consistency and stability of these preparations in various batches that are essential in enhancing reproducibility in animal research. Among the major parameters of physicochemical characteristics are total ash (usually 5 or less), insolubility of ash in acids, insolubility of ash in acids, extraction value in the product of water and alcohol, and a pH value within the optimal set of parameters (5.0 to 7.0). These parameters are quantitative measures of purity, moisture content and all-round stability of formulation.

In a single preclinical anti-ulcer study on *Glycyrrhiza glabra* (Licorice), *Embllica officinalis* (Amla) and *Zingiber officinale* (Ginger), physicochemical consistency was well related to the enhancement of the pharmacological effects in Wistar rats. The experiment proved that batches with the best physicochemical requirements had the highest decrease of ulcer index, which states that the standardization of chemical composition has a direct proportional impact on therapeutic performance. Equally, qualitative phytochemical screening validated the existence of active classes flavonoids, tannins, and glycosides of which have synergistic effect on the onset of pharmacodynamics. All of these findings prove that physicochemical and phytochemical homogeneity develops a scientific ground of the connection between composition and efficacy<sup>15</sup>.

### 3.3. Chromatographic Fingerprinting

Chromatographic profiling has become a fundamental method used in the standardization of polyherbal preparations in providing the level of high accuracy in accounting chemical identity and adulteration detection. High-Performance Liquid Chromatography (HPLC) and High-Performance Thin Layer Chromatography (HPTLC) are the techniques that allow observing the unique chemical fingerprints with the great number of individual peaks associated with active phytoconstituents<sup>16</sup>.



**Figure 2:** Chromatographic Fingerprinting<sup>17</sup>

In case, analysis by HPTLC of a polyherbal hepatoprotective formulation showed that specific peaks of andrographolide (*Andrographis paniculata*) and berberine (*Tinospora cordifolia*) were directly correlated with the normalization of liver enzyme levels to diuretic levels namely; ALT, AST, and ALP in rat models of paracetamol induced hepatotoxicity. This association of chromatographic purity to the pharmacodynamic effect illustrates the significance of chemical fingerprinting to the creation of stable therapeutic performance. Moreover, the chromatographic prints can be used as important references points of quality control in the regulation because the batches of production are supposed to be of the same chemical structure but with different properties. Therefore, chromatographic validation does not only nevate herbal identity but it also gives a measurable correlation between analytic indicators and biological response<sup>18</sup>.

### 3.4. Animal-Based Pharmacological Validation

Pharmacological validation using animals is critical in substantiating bio-relevance of biological findings in pharmacognosics and analytics. The relevance of standardized formulations in relation to their physiological and therapeutic value can thus be evaluated successfully through controlled studies in vivo. An example is the observation that polyherbal preparations consisting of antioxidants such as *Terminalia chebula* (Haritaki) *Curcuma longa* (Turmeric) and *Camellia sinensis* (Green tea) have a significant dose-dependent enhancement of endogenous antioxidant enzyme levels; superoxide dismutase (SOD), catalase, and glutathione peroxidase, in rat liver homogenates. These beneficial changes in biochemicals indicate that the standardized occurrence of phenolic and flavonoid compounds is directly correlated with the antioxidant activity<sup>19</sup>.



**Figure 3:** Animal Pharmacological<sup>20</sup>

Equally, polyherbal preparations of antidiabetic agents, which were administered on streptozotocin induced diabetic rat, were found to generate considerable effects of reducing the level of fasting blood glucose and increasing the storage of hepatic glycogen and insulin-sensitivity. These results confirm the physiological consequences due to standardization solutions that are pharmacognostically and chemically standardized which affirm the validity of the standardization method. Further, the pharmacological models of animals offer a working ground into the study of dose-response correlations, toxicity pattern, and the mechanistic effects- therefore creating an elaborate scientific framework in which a traditional herbal knowledge system intersects pharmacological confirmations<sup>21</sup>.

#### 4. EXPERIMENTAL APPROACHES IN ANIMAL MODELS

Polyherbal formulations are reliable, safe, and effective due to the use of appropriate animal models, standard dosing and thorough pharmacological analysis. These approaches connect standardization of pharmacognostic to biological measurable effects giving scientific support to the traditional therapeutic assertions<sup>22</sup>.

##### Selection of Animal Models

The identification of suitable animal models is also a fundamental part of the experimental pharmacognosy since directly affects the reliability and translational power of the pharmacological results. The rodent most commonly used include rats of the Wistar rats, Sprague Dawley rats and Swiss albino mice as they are physiologically comparable to humans, convenient to manipulate, cost effective and well documented genetics and other metabolic attributes. Reproducible biological reactions are also made available by these models, and this is needed to determine the safety and efficacy of polyherbal preparations properly<sup>23</sup>.

The animal model selection is dependent on the targeted pharmacological endpoint of interest. As an example the carrageenan induced paw edema or formalin induced paw edema model in rats are generally used to assess the anti-inflammatory activity, and recreates similar acute inflammatory effects on animals. The models used in hepatoprotective research typically comprise chemically induced liver disease models (e.g. paracetamol or carbon tetrachloride (CCl<sub>4</sub>) intoxication) and lead to the measurement of enzyme restoration and tissue regeneration. Likewise, antioxidant and anti-stress researches apply oxidative stress inducing agents or anti-restraint stress paradigms to measure biochemical and behavioral changes. Due to these specific animal models, there is good experimental justification of connecting pharmacognostic standardization to pharmacological efficacy, and thus, they are essential in polyherbal validation experiments.

##### Dose Standardization and Administration

Proper dosage is essential to guarantee therapeutic as well as safety in preclinical pharmacology testing. Polyherbal formulations are dose-standardized based on international OECD guidelines, in particular OECD 423 (Acute Oral Toxicity) and OECD 407 (Repeated Dose 28-Day Oral Toxicity). These procedures allow the researchers to determine the median lethal dose (LD<sub>50</sub>)

and the level of no adverse effect (NOAEL), which is the basis of safe and effective therapeutic doses<sup>24</sup>.

Polyherbal extracts are usually taken by the mouth, as a reflection of the most common clinical route of entry in humans. The extract is suspended in any of the appropriate vehicles like distilled water, 0.5% carboxymethyl cellulose (CMC) or the hydroalcoholic solutions to allow suitable dosage and uniform absorption. The range of experimental doses is 100-1000 mg/kg body weight, which varies with the formulation, the active constituents and their concentration. Administration is 7 to 28 days, which provides the opportunity to observe both the acute pharmacodynamic reactions, as well as the sub-chronic toxicity effect. This systematic strategy is used to ensure that dosage regimes are always biologically relevant, and as well as avoid the possible toxic effects brought about by herbal over-exposure or synergy of various plant components<sup>25</sup>.

### Pharmacological and Biochemical Evaluation

Pharmacological and biochemical evaluation phase is the ultimate assessment of the effectiveness and safety of a polyherbal formulation that puts the analytical standardization into transparency into the biological significance. Such assessments include behavioral, biochemical and histopathological assessments in order to affirm therapeutic claims under regulated conditions<sup>26</sup>.

- **Anti-inflammatory Activity:** It is commonly measured applying different paw edema models with carrageenan or formalin administration into rats, where the volume of the paw decreases, and it is treated as an indication that inflammation is inhibited. Reference drugs like indomethacin or diclofenac are used in comparison with standardized formulations in order to measure their corresponding efficacy<sup>27</sup>.
- **Antioxidant Potential:** The antioxidant assays measures the rise in essential body antioxidants Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the critical body organs like the liver and kidney. The high enzyme levels indicate the improved free radical scavenging, which justifies the role of the formulation as an oxidative stress protector.
- **Hepatoprotective Assessment:** This is assessed by use of paracetamol or CCl<sub>4</sub> induced hepatotoxicity models. Important restoration of hepatic integrity is indicated by significant normalization of serum biomarkers; Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in combination with an improved histopathological liver architecture.
- **Analgesic and Anti-stress Activities:** Behavioral models used include hot plate test, tail-flick test, and forced swim test which determine the analgesic and adaptogenic action of the formulations. The time-based action of decreased latence in responses to pain and longer immobility in stress states demonstrate have been modified in the central nervous system and ability to resist stress<sup>28</sup>.

Such a well-developed pharmacological profiling will guarantee that every biological assertion of a polyherbal manufacturing is supported by quantitative data, thus, determining its therapeutic efficacy and safety profile<sup>29</sup>.

## 5. DISCUSSION

Efficacy along with safety and authenticity of polyherbal formulations will be guaranteed by the combination of pharmacognostic standardization and the animal-based validation that will establish the safety, accuracy of analytical examination, and biological output<sup>30</sup>. The way forward into research work is focusing on global standardization, improved analysis tools and pharmaceutical integration, to improve reproducibility and acceptance of herbal medicines by the regulators<sup>31</sup>.

### 5.1. Interpretation and Analysis of Findings:

The overall bodies of evidence derived on a basis of animal research are categorical that pharmacognostic standardization forms the basis of ensuring truthfulness, purity, and dependability of poly-herbal preparations. It has been shown in the course of preclinical testing in rodent models that formulations standardized by using macroscopic, microscopic, physicochemical, and chromatographic analyses have similar and predictable therapeutic responses<sup>32</sup>. The high level of dependence between both physicochemical homogeneity (as determined by the value of ash, pH and extractive yields), and pharmacological action supports the importance of compositional homogeneity. In addition, chromatographic fingerprinting and phytochemical profiling have been found to be instrumental in verification of plant identity and association of bioactive components to observed effects of pharmacological properties, including anti-inflammatory, hepatoprotective and antioxidant effects, such that functional relevance of analytical standardization is confirmed<sup>33</sup>.

### 5.2. Implications and Significance:

The results of this study emphasize the importance of the combined use of pharmacognostic and preclinical validation as a bridge between traditional and modern pharmacological sciences<sup>34</sup>. The proven connection between analytical accuracy and biological effectiveness of polyherbal formulations underlines the significance of the implementation of homogenous quality control modalities of polyherbal formulations to create international viability and regulatory acceptance<sup>35</sup>. There is scientific complexity in which animal research can be used to assess the safety, toxicity, and mechanism of action to increase the perception of herbal therapies in the field of evidence-based medicine<sup>36</sup>. Moreover, animal data combined with chromatography and phytochemical markers greatly helps in setting the reference standards and pharmacopoeial records, which are the stepping stones to the industrial manufacturability and quality assurance of herbal drugs<sup>37</sup>.

### 5.3. Gaps and Future Research Directions:

Although there have been tremendous improvements, issues still exist because there are no harmonized international standards, inter-laboratory differences, or searching of pharmacodynamics and pharmacokinetics of interactions of individual herbs<sup>38</sup>. To address these

gaps, additional research would need to work on the creation of internationally agreed standards in the pharmacognostic standardization using the latest analytical techniques including the LC-MS, NMR, GC-MS, and metabolomics to provide further chemical and bioavailability analysis<sup>39</sup>. Correlations between and phytochemical fingerprints and pharmacological activity in animal models can be reinforced with the addition of bioinformatics and chemometric modeling. Also, the traceability, reproducibility, and translational capabilities will be improved by developing digital databases of standardized formulation and performing long-term toxicity and mechanism-based animal research. To incorporate all the information presented thus far, it can be well concluded that pharmacognostic standardization when combined with animal-based validation provides a strong scientific basis on how to convert polyherbal preparations into therapeutic tools that are internationally accepted as safe and effective<sup>40</sup>.

## 6. CONCLUSION

The present review illustrates that meticulous pharmacognostic standardization (i.e., a combination of macroscopic/microscopic authentication, physicochemical and phytochemical profiling, and chromatographic fingerprinting) when coupled with properly designed animal-based pharmacological assessments can establish a reliable and reproducible basis of the quality assurance of polyherbal preparations; consistently reproducible all-important anti-inflammatory, hepatoprotective, antioxidant and antidiabetic actions in animal models and allow. However, their continued use has been challenged by contradictory aspects of inter-laboratory variability, lack of world guidelines, insufficient use of sophisticated omics/analytical systems and little research on herb-herb pharmacokinetics/pharmacodynamics that need to be overcome in terms of regulatory acceptance and clinical translation. Thus, the implementation of the standardized pharmacognostic-analytical protocols, the introduction of the modern LC-MS/NMR/metabolomics and chemometric methods and the development of the validated digital reference libraries and the broadening of the long-term and mechanism-based animal models are the necessary further steps to convert traditional polyherbal preparations into evidence-based globally recognized therapeutics.

## Acknowledgment

We extend our sincere gratitude to Sri Ganganagar College of Ayurvedic Science and Hospital, Sri Ganganagar, Rajasthan 335002, for their invaluable support and contribution to Ayurvedic education and research. Their commitment to preserving traditional knowledge while fostering modern clinical practices has significantly enriched our understanding and exploration of holistic healthcare. We especially acknowledge the faculty and staff whose dedication continues to inspire excellence in the field of Ayurveda.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

**REFERENCES**

1. Agarwal, P., Goyal, A., & Vaishnav, R. (2018). Comparative quality assessment of three different marketed brands of Indian polyherbal formulation-triphala churna. *Journal of Biomedical Research*, 2, 9.
2. Animish, A., & Jayasri, M. A. (2023). Standardization and Quality Control Analysis of a Polyherbal Antidiabetic Formulation. *Current Trends in Biotechnology and Pharmacy*, 17(Supplement 3B), 1252-1270.
3. Ashraf, N., Naikodi, M. A. R., Kumar, G. S., Ahmed, T., & Iftikhar, Y. (2024). Physicochemical standardization, evaluation of antioxidant and antimicrobial activity of a polyherbal formulation. *Annals of Phytomedicine*, 13(2), 1-8.
4. Bidikar, C. M., Hurkadale, P. J., Nandanwadkar, S. M., & Hegde, H. V. (2022). A validated spectro densitometric regulatory compliant USP-HP-TLC protocol for quantification of polyphenols and antioxidants from polyherbal formulations containing Terminalia species. *Journal of Chromatography B*, 1207, 123379.
5. Boini, T., Misro, L., Maurya, R., KS, R., Radhakrishnan, T., Sharma, P., ... & Acharya, R. (2025). Simultaneous quantification of five bioactive markers for standardization of ayurvedic polyherbal formulation Jwarahara Kwatha Choornam using HPTLC. *Scientific Reports*, 15(1), 33023.
6. Devi, U., Ahmed, A., Ansari, S. A., Khan, A. S., & Meena, R. (2024). Pharmacognostic standardization and quality control of stem bark of *Cinnamomum cassia* (L.) J. Presl. *Asian Journal of Pharmaceutical Research and Development*, 12(3), 75-83.
7. Dinakaran, S. K., Chelle, S., & Avasarala, H. (2019). Profiling and determination of phenolic compounds in poly herbal formulations and their comparative evaluation. *Journal of traditional and complementary medicine*, 9(4), 319-327.
8. Dinakaran, S. K., Sujiya, B., & Avasarala, H. (2018). Profiling and determination of phenolic compounds in Indian marketed hepatoprotective polyherbal formulations and their comparative evaluation. *Journal of Ayurveda and integrative medicine*, 9(1), 3-12.
9. Gupta, M. K., Khade, M. A., Srivastava, B., Hyam, S. R., & Gurav, P. B. (2022). Development of a Gastroretentive Polyherbal Formulation and its Standardization. *Pharmacognosy Research*, 14(4).
10. Gupta, P., Pal, A., Trivedi, S., & Gupta, R. K. (2021). Review on quality control parameters for standardisation of herbal drug. *Journal of Advanced Scientific Research*, 12(03), 35-41.
11. Haque, M. R., Tara, N., & Nandi, M. (2020). Pharmacognostical, physico-chemical and contamination studies of polyherbal formulation "Qurse pudina". *Trends in Phytochemical Research*, 4(3), 153-164.
12. Harinarayanan, C. M., Deepak, M., Geetha, S. P., Karnwal, A., Kumar, G., Kumar, A., & Malik, T. (2024). Pharmacognostic Characterization of Panchatikta, an Ayurvedic Polyherbal Combination—Exploring the Role of Botanical and Phytochemical Techniques in the Standardization and Validation of Traditional Wisdom. *Advances in Pharmacological and Pharmaceutical Sciences*, 2024(1), 9238271.
13. Hartanti, D., Chatsumpun, N., Sa-Ngiamsumtorn, K., Supharattanasitthi, W., Kitphati, W., & Peungvicha, P. (2023). The pharmacognostic standards, antioxidant and antidiabetic activities,

- and hepatic safety profile of an Indonesian antidiabetic polyherbal formulation. *Indonesian Journal of Pharmacy*, 65-78.
14. Joshi, A., Thakar, A., & Harisha, C. R. (2022). PHARMACOGNOSTICAL AND PHARMACEUTICAL ANALYSIS OF PANCHATIKTAGHRITA GUGGLU AN AYURVEDIC POLYHERBAL FORMULATION. *World J Pharm Res*.
  15. Kumar, J., & Bajpai, T. (2025). Standardization and Quality Control of Polyherbal Formulations Using HPLC and UV-Vis Spectroscopy. *International Journal of Pharmacognosy and Herbal Drug Technology*, 194-205.
  16. Kumar, M. M., Sharma, A., Rinka Juneja, D. T. P., Arif, M. M., Singh, M. A. D., & Kumar, A. (2025). Biological Evaluation Of Herbal Preparations And Formulation Standardization: A Literature Study. *Journal of Neonatal Surgery*, 14(8s).
  17. Kumar, V., Kushwaha, V., Charde, V., Jagtap, C., Gandhi, Y., Grewal, J., ... & Dhiman, K. S. (2022). The validated pharmaceutical standard operating procedure and quality control study of the coded polyherbal tablet formulation AYUSH SG-5. *South African Journal of Botany*, 151, 319-327.
  18. Lijima, C., Dei, L., Harisha, C. R., & Shukla, V. J. (2023). Pharmacognostical and pharmaceutical analysis of Jeevantyadi Avaleha: A polyherbal Ayurvedic formulation. *Journal of Drug Research in Ayurvedic Sciences*, 8(2), 150-158.
  19. Metri, S., Gopari, S., & Mathew, C. (2025). Qualitative and Quantitative Standardization of Liv. 52: A Polyherbal Formulation: Standardization of Liv. 52: A Polyherbal Formulation. *Journal of Ayurveda and Integrated Medical Sciences*, 10(7), 66-72.
  20. Misro, L., Boini, T., Maurya, R., Radhakrishnan, T., Rohith, K. S., Kumar, V., ... & Acharya, R. (2024). Analytical method development and validation for simultaneous estimation of seven markers in polyherbal formulation JKC by using RP-HPLC. *Future Journal of Pharmaceutical Sciences*, 10(1), 92.
  21. Nainwal, P. A. N. K. A. J., & Singh, N. A. R. D. E. V. (2019). Quality control evaluation of in-house prepared polyherbal ayurvedic formulation saptavimsatika guggulu. *Asian J Pharm Clin Res*, 12(5), 317-320.
  22. Neethu, S., Veena, S. K., Indulekha, V. C., Eapen, J., & Radhakrishnan, K. V. (2021). Phytoconstituents assessment and development of standardization protocol for 'Nayopayam Kwatha', a polyherbal Ayurvedic formulation. *Journal of Ayurveda and integrative medicine*, 12(3), 489-499.
  23. Paliwal, S. (2020). STANDARDIZATION AND EVALUATION OF TWO MARKETED POLYHERBAL FORMULATION (GUTIKAS). *Asian J Pharm Clin Res*, 13(9), 81-83.
  24. Parganiha, R. (2025). Comparative Pharmacognostic Evaluation and Standardization of Root Extracts of Selected Ayurvedic Plants. *Interconnected Journal of Chemistry and Pharmaceutical Sciences (IJCPS)*, 18-35.
  25. Patel, A., Hirani, K., Bhuvu, V., & Panchal, P. (2020). Assessment of quality control parameters and standardization of peppard syrup: a polyherbal formulation. *International Ayurvedic Medical Journal*, 8(4), 3620-3627.
  26. Patel, J. (2025). Preparation and Standardization of some herbal Antidiabetic drugs in Polyherbal Formulation. *Asian Journal of Pharmaceutics (AJP)*, 19(01).

27. Patil, S. C., Baghel, A. S., Kamble, S. B., Rudrappa, H. C., Shukla, V. J., & Mehta, M. D. (2022). Pharmacognostical and physicochemical profile of Abhayadi Gutika: An polyherbal formulation. *Journal of Indian System of Medicine*, 10(4), 221-227.
28. Pawar, D., Manjusha, R., Harisha, C. R., & Shukla, V. J. (2020). PHARMACOGNOSTICAL AND PHYSICO-CHEMICAL EVALUATION OF TUNGADRUMADI TAILA-AN AYURVEDIC POLYHERBAL FORMULATION.
29. Qazaf, M., Naureen, H., shabir Ahmed, M., & Ahsan, M. A. (2021). Pharmacognostic analysis and antidiabetic potential of selected marketed polyherbal formulations. *Journal of Bashir Institute of Health Sciences*, 2(2), 43-54.
30. Rezghi, M., Choopani, R., Fahimi, S., Sheihkoleslami, M. A., & Hamzeloo-Moghadam, M. (2019). Formulation of a traditional polyherbal product to a standard pharmaceutical syrup and development of its quality control methods. *Research Journal of Pharmacognosy*, 6(4), 53-59.
31. Sabari Sengupta, P., Thakar, A., Harisha, C. R., & Shukla, V. J. (2019). Pharmacognostical and analytical study of Kushmanda ghrita, a polyherbal formulation, for pharmaceutical standardization.
32. Sahare, A. Y., Akare, S. C., Agrawal, R. B., Gotephode, S. N., & Gajbhiye, S. A. (2025). Standardization and Quality Control of Ayurvedic Polyherbal Formulations. *International Journal of Pharmacognosy and Herbal Drug Technology*, 103-120.
33. Sharma, P., More, A. B., Galib, R., Rai, S., & Upadhyay, A. (2024). Pharmacognosy and Analytical Profile of Bhunimbadi Churna: A Polyherbal Formulation. *Indian Journal of Ayurveda and Integrative Medicine KLEU*, 5(1), 25-30.
34. Shyamkant, C. D., Chavan, M. J., & Upasani, C. D. (2018). Standardization of Jambavasava-a polyherbal ayurvedic formulation. *Asian Journal of Pharmaceutical Research and Development*, 43-46.
35. Srivastava, S., & Misra, A. (2018). Quality control of herbal drugs: Advancements and challenges. In *New age herbals: resource, quality and pharmacognosy* (pp. 189-209). Singapore: Springer Singapore.
36. Subba, A., & Mandal, P. (2019). Standardization Of A Traditional Polyherbal Formulation With Pharmacognostic Study; Its Phytochemical Content, Antioxidant, And Antidiabetic Activity. *Asian J Pharm Clin Res*, 12(5), 182-190.
37. Unnikrishnan, D., Choudhary, P., & Sukumaran, S. K. (2024). Pharmacognostical and physicochemical evaluation of Prajasthapana granules: A novel polyherbal Ayurvedic formulation. *Journal of Drug Research in Ayurvedic Sciences*, 9(6), 417-425.
38. Unnikrishnan, D., Choudhary, P., & Sukumaran, S. K. (2024). Pharmacognostical and physicochemical evaluation of Prajasthapana granules: A novel polyherbal Ayurvedic formulation. *Journal of Drug Research in Ayurvedic Sciences*, 9(6), 417-425.
39. Vats, S., Agarwal, K., & Kumar, A. (2022). Preparation and standardization of Vrihatsamsharkar Churna: a polyherbal formulation. *Research Journal of Pharmacy and Technology*, 15(5), 2244-2249.
40. Vijapur, L. S., Singh, G., Pandey, J., Patel, B. A., Shyamala, J. K., Krishna, K. V. V., ... & Tiwari, G. (2024). Formulation standardization and quality control of polyherbal formulation for treatment of type 2 diabetes mellitus. *Nanotechnol Percept*, 20(11), 775-783.