



REVIEW ARTICLE

## STANDARDIZATION, PHYTOCHEMICAL PROFILING, AND PHARMACOLOGICAL VALIDATION OF POLYHERBAL FORMULATIONS FOR AUTO-INFLAMMATORY AND ANALGESIC APPLICATIONS

Abhay Kumar Mourya<sup>\*1</sup>, Manju Makhija<sup>1</sup>, Shanti Bhushan Mishra<sup>2</sup>, Mahesh Gupta<sup>1</sup>

School of Health & Allied Sciences<sup>1</sup>, Career Point University, Kota, Rajasthan, India.  
United College of Pharmacy<sup>2</sup>, Naini, Uttar Pradesh, India.

### Article History

Received: 07 July 2025  
Revised: 28 July 2025  
Accepted: 13 August 2025  
Published: 25 September 2025

### Correspondence should be addressed to

Abhay Kumar Mourya,  
PhD. Research Scholar,  
School of Health & Allied Sciences,  
Career Point University, Kota,  
Rajasthan, India.

Email- abhaymourya.bph@gmail.com

**ABSTRACT:** The increasing burdens of the world with inflammatory diseases and chronic pains have enhanced the need to have safer, multi-targeted and more effective therapeutic approaches. Polyherbal preparations with their deep-rooted involvement in the traditional systems of medicine are beginning to be viewed as innovative solutions because of their synergetic interactions and holistic action mechanisms. In comparison to the traditional pharmacotherapies, like non-steroidal anti-inflammatory drugs and opioids, which have many side effects, tolerance, and long-term toxicity, polyherbal combinations present a better effect with higher quality and fewer adverse effects and patient adherence. This review is concerned with giving a comprehensive focus on the standardization, phytochemical profiling, and pharmacological validation of polyherbal formulations that have been designed towards the use of anti-inflammatory and analgesic purposes. Phytochemical studies are detailed describing a wide range of bioactive secondary metabolites; flavonoids, alkaloids, phenolic compounds, terpenoids, saponins, among others, that all play a role in their therapeutic efficacy. The quality assessment, fingerprinting, and reproducibility of these complex herbal matrices have been transformed by the use of advanced analytical tools namely HPTLC, HPLC and GC-MS. Pharmacological analyses using *in vitro* and *in vivo* models indicate high modulation of inflammatory pathways, blockage of pro-inflammatory products, control of cytokines, and inhibition of oxidative stress pathways. Moreover, such formulations possess the ability to act on a variety of signaling pathways in isolation or in combinations, increasing the therapeutic outcome. Nevertheless, such issues as absence of global standardization procedures, variability of phytochemicals, and the absence of clinical approval are still considerable. The way forward focuses on merging the technology of omics with systems biology and on sound clinical trials to support scientific validity, and provide the conversion of the polyherbal preparations into conventionalized, evidence-based medicine to the contemporary healthcare framework.

**Keywords:** Polyherbal formulation, Standardization, Phytochemical profiling, Anti-inflammatory, Analgesic, Herbal medicine

## I. INTRODUCTION

Inflammation and pains are critical biological reactions that prevent tissue damages, infection, and noxious stimuli, and act as protective responses. Acute inflammation is a localized and very rapid reaction, which is marked by redness, swelling, heat and pain and which is used to get rid of the primary cause of cell damage and to begin the process of repairing the tissues [1]. On the contrary, chronic inflammation is an unbalanced and long-term process that is also associated with the emergence of other pathological disorders, such as rheumatoid arthritis, cardiovascular diseases, neurodegenerative disorders, and autoimmune syndromes. Pain, which frequently accompanies inflammation, may be categorized as acute or chronic, and it may have a serious effect on the physical functioning, emotional state, and the general quality of life. Although significant progress has been made regarding pharmacotherapy, the treatment of inflammation and pain is complicated and ineffective in long-term clinical conditions [2].

Traditional treatment agents, especially non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, are actively used in treating inflammatory disorders and pain syndromes. Although these drugs have a symptomatic effect, long-term risk or excessive medication has serious adverse effects that include gastrointestinal ulceration, renal impairment, cardiovascular risks, tolerance, dependence as well as addiction potential [3]. These restrictions underscore the importance of seeking safer, more effective and sustainable therapeutic options that will help in dealing with the efficacy and safety issues. Here, herbal medicine has come back to the limelight as a useful source of therapeutic agents, and polyherbal preparations are receiving growing scientific and clinical interest [4]. Polyherbal preparations, which form the foundation of traditional medicine systems like Ayurveda, are the balanced blending of several medicinal plant preparations to realize great therapeutic effect due to synergistic arrangements. This synergism lets simultaneous modulation of several biological pathways leading to better pharmacological

responses and lower toxicity levels than those of individual compounds [5].

These formulations are phytochemically abundant in various bioactive compounds such as flavonoids, alkaloids, phenolic compounds, terpenoids, and glycosides which combine to make the formulations have anti-inflammatory and analgesic effects. The problem, however, is that the nature and variability of polyherbal systems are extremely difficult in terms of quality control, reproducibility, and acceptance by regulatory bodies [6]. The differences in the sources of plants, the conditions of harvesting, the modes of extracting the products, and the procedures of the formulation may result in differences in the therapeutic effects. Hence, polyherbal formulations need to be subjected to systematic standardization and full pharmacological validation in order to guarantee the safety, effectiveness and operation of these preparations [7]. In this connection, new methods of analysis and experimental designs are especially important in making a scientific credibility. The current review seeks to critically examine the current developments in standardization, phytochemical profiling, and pharmacological validation of poly herbal preparations against anti-inflammatory and analgesic purposes as well as discussing current problems and future research directions [8].

## 2. Literature Search Methodology

The literature search was carried out in a systematic and comprehensive manner in order to gather the scientific facts on standardization, phytochemical profiling and pharmacological validation of polyherbal formulations with anti-inflammatory and analgesic properties. Several electronic databases such as PubMed, Scopus, ScienceDirect, and Google Scholar, were thoroughly searched to be able to cover a wide range of peer-reviewed research articles, reviews, and experimental studies [9]. The search strategy was aimed at capturing both the old and the new developments in the field.

Search was narrowed down to appropriate keywords and combinations which consisted of: polyherbal formulations, anti-inflammatory, analgesic, phytochemical profiling, standardization and herbal medicine. The relevance and specificity of the retrieved literature was used by employing the use of the Boolean operators like AND and OR. Furthermore, reference lists of articles of particular interest were cursory screened to find additional relevant studies, which could not be detected in the initial search [10].

The inclusion criteria were specified in such a way that scientifically strong and relevant studies were selected. Articles that reported *in vitro*, *in vivo*, and clinical trials of the polyherbal formulations with anti-inflammatory and analgesic properties were accepted. Papers that dealt with phytochemical analysis, standardization methods and pharmacological validation were also taken into consideration. Articles published in the English language and having full-texts were considered to keep consistency and clarity in the interpretation of data [11].

The exclusion criteria were that the studies had to be experimentally validated, their methodology had to be fully described or the study was to be on a single herbal extract and not on a polyherbal system. Other exclusion criteria were also abstracts containing no full texts and duplications of studies. The chosen articles were critically evaluated and the appropriate data were obtained and systematically tabulated. It focused on the study design, the type of formulation, phytochemical constituents, analytical procedures, pharmacological models and important outcomes. Information was synthesized to come up with a consistent and evidence-based review of the topic [12].

## 3. Concept of Polyherbal Formulations

Polyherbal preparations are a central principle of the traditional medicine systems, especially Ayurveda, in which the so-called synergism is underlined. Synergism is used to describe the effect of several medicinal plants, where the effect of the combination of the plants is higher than that of the individual effect [13]. Polyherbal formulations unlike single-compound therapies are aimed at the multisimultaneous targeting of several pathways and thus providing a holistic approach to disease management. This combination approach not only increases the treatment efficacy but also assists in decreasing unwanted side effects of single drug high doses.

Multi-target action is one of the greatest benefits of polyherbals. There are various biochemical pathways that are involved in inflammatory and pain-related disorders, these include release of cytokines, production of prostaglands, oxidative stress and immune modulation. Polyherbal preparations have the capacity to work on these various pathways simultaneously resulting in a better and all-rounder management of the condition [14]. Moreover, the complementary and supportive effects are also possible since these preparations contain different bioactive constituents. A second important advantage is decreased toxicity. Various plant constituents tend to have a combination effect resulting in lower individual dosages, which reduces the chances of side effects and makes the safety profiles better. Moreover, some elements in the formulation can offset the toxicity of other ones, which makes the therapeutic system much safer [15]. There is also improved efficacy with polyherbal formulations as there is pharmacodynamic and pharmacokinetic interaction between the constituents. The interactions can enhance bioavailability, stability and absorption of active compounds that results in better clinical outcomes [16].

Polyherbal preparations often incorporate a number of medicinal plants due to their strong anti-inflammatory and pain-relieving effects. Curcumin is highly anti-inflammatory and antioxidant, and abundant in *curcuma longa* (turmeric). *Withania somnifera* (Ashwagandha) has an immunomodulatory and adaptogenic effect and *Zingiber officinale* (ginger) is broadly acknowledged to have an analgesic and anti-inflammatory effect. The effective polyherbal therapeutic systems are based on the rational combination of such plants (Table 1) [17].

**Table 1: Common Medicinal Plants Used in Polyherbal Anti-inflammatory and Analgesic Formulations [18]**

S. No.	Plant Name	Family	Major Constituents	Pharmacological Role
1	<i>Curcuma longa</i>	Zingiberaceae	Curcumin	Anti-inflammatory, antioxidant
2	<i>Withania somnifera</i>	Solanaceae	Withanolides	Immunomodulatory, anti-inflammatory
3	<i>Zingiber officinale</i>	Zingiberaceae	Gingerols, shogaols	Analgesic, anti-inflammatory
4	<i>Boswellia serrata</i>	Burseraceae	Boswellic acids	Anti-inflammatory
5	<i>Tinospora cordifolia</i>	Menispermaceae	Tinosporaside, alkaloids	Immunomodulatory, anti-inflammatory
6	<i>Azadirachta indica</i>	Meliaceae	Nimbin, azadirachtin	Anti-inflammatory, antimicrobial
7	<i>Ocimum sanctum</i>	Lamiaceae	Eugenol, ursolic acid	Anti-inflammatory, antioxidant
8	<i>Glycyrrhiza glabra</i>	Fabaceae	Glycyrrhizin	Anti-inflammatory, soothing effect
9	<i>Piper nigrum</i>	Piperaceae	Piperine	Bioavailability enhancer, analgesic
10	<i>Allium sativum</i>	Amaryllidaceae	Allicin	Anti-inflammatory, antioxidant
11	<i>Aloe vera</i>	Asphodelaceae	Aloin, polysaccharides	Anti-inflammatory, wound healing
12	<i>Centella asiatica</i>	Apiaceae	Asiaticoside	Anti-inflammatory, tissue repair

#### 4. Phytochemical Profiling of Polyherbal Formulations

A phytochemical profiling will be an important step in determining pharmacological potential of polyherbal preparations. They are complex mixtures of bioactive secondary metabolites of various plant sources and all these are contributions to their pharmacological effects. Extensive phytochemical assessment is useful in determining the active constituents as well as in the regulation, standardization, and reproducibility of herbal preparations. Plant variability in origin, extraction and formulation processes require systematic profiling to be guaranteed in terms of uniformity and effectiveness [19].

##### 4.1 Preliminary Phytochemical Screening

The initial phytochemical screening is a qualitative analysis to identify the existence of the major classes of secondary metabolites like alkaloids, flavonoids, tannins, saponins, terpenoids and glycosides in polyherbal formulations. It is these compounds that mainly have biological activity that is attributed to the herbal medicines [20].

Basic qualitative testing is commonly used as a screening tool. As an example, alkaloids are determined by the reagents of Dragendorff and Mayer, flavonoid by the Shinoda test, tannin by the ferric chloride test, and saponin by the foam test. Such simple, quick, cheap techniques give a preliminary idea about the phytochemical structure and aids in the choice of appropriate extracts to continue with more detailed research. These tests are preliminary, but they provide grounds on which higher analytical studies are conducted [21].

#### 4.2 Major Bioactive Constituents

Polyherbal formulations are highly endowed with a wide range of bioactive compounds, which assume synergistic pharmacological effects. Flavonoid is one of the most significant groups of them because of their strong effect as anti-inflammatory agents, which is mainly related to the inhibition of pro-inflammatory enzymes and cytokines. Another important group of substances is terpenoids, which have analgesic and anti-inflammatory effects, and usually the inhibition of pain signaling pathways [22].

Phenolic compounds also play a significant role in the antioxidant effect of polyherbal preparations in the form of scavenging of free radicals and alleviating oxidative stress, which is a major cause of inflammation and tissue injury. Moreover, alkaloids, as well as saponins, can be also used as supportive agents because they can improve bioavailability of active constituents and immune responses. Polyherbal formulations are able to activate on several biological targets at the same time due to the combined presence of these compounds [23].

#### 4.3 Analytical Techniques

Proper identification, quantification, and standardization of phytoconstituents contained in polyherbal preparations are impossible without advanced methods of analysis. HPTLC is a modern profiling technique employed to analyse and compare herbal extracts and also in profiling fingerprints. It gives a visualization of the various compounds and it comes in handy especially with the routine quality control [24].

**Table 2: Phytochemical Constituents Identified in Polyherbal Formulations [27]**

S. No.	Formulation Type	Plant Components	Extraction Solvent	Major Phytochemicals Identified	Analytical Method
1	Polyherbal mix A	<i>Curcuma longa</i> , <i>Zingiber officinale</i>	Ethanol	Curcumin, gingerols, flavonoids	HPTLC
2	Polyherbal mix B	<i>Withania somnifera</i> , <i>Tinospora cordifolia</i>	Methanol	Withanolides, alkaloids, phenolics	HPLC
3	Polyherbal mix C	<i>Boswellia serrata</i> , <i>Glycyrrhiza glabra</i>	Ethanol	Boswellic acids, glycyrrhizin	GC-MS
4	Polyherbal mix D	<i>Ocimum sanctum</i> , <i>Azadirachta indica</i>	Aqueous	Eugenol, nimbin, flavonoids	HPTLC
5	Polyherbal mix E	<i>Piper nigrum</i> , <i>Allium sativum</i>	Methanol	Piperine, allicin	GC-MS
6	Polyherbal mix F	<i>Aloe vera</i> , <i>Centella asiatica</i>	Aqueous	Aloin, asiaticoside, saponins	HPLC
7	Polyherbal mix G	<i>Curcuma longa</i> , <i>Boswellia serrata</i>	Ethanol	Curcumin, terpenoids	LC-MS
8	Polyherbal mix H	<i>Withania somnifera</i> , <i>Ocimum sanctum</i>	Methanol	Withanolides, phenolics	HPTLC
9	Polyherbal mix I	<i>Tinospora cordifolia</i> , <i>Azadirachta indica</i>	Aqueous	Alkaloids, limonoids	HPLC
10	Polyherbal mix J	<i>Zingiber officinale</i> , <i>Piper nigrum</i>	Ethanol	Gingerols, piperine	GC-MS
11	Polyherbal mix K	<i>Glycyrrhiza glabra</i> , <i>Allium sativum</i>	Methanol	Glycyrrhizin, sulfur compounds	LC-MS
12	Polyherbal mix L	<i>Centella asiatica</i> , <i>Aloe vera</i>	Aqueous	Asiaticoside, polysaccharides	HPTLC

The high-performance liquid chromatography (HPLC) provides accurate quantification of individual compounds of

the markers and is used to standardize them. The gas chromatography-mass spectrometry (GC-MS) is very useful in

determining volatile as well as semi volatile compounds, particularly essential oils and terpenoids. Also, liquid chromatography mass spectrometry (LC-MS) offers extremely sensitive and precise characterization of complicated phytochemical mixtures, which allows the structural clarification of the obscure compounds (Table 2) [25].

Combined with other analytical methods, extensive phytochemical outlining becomes thinkable, which growths the reliability, safety, and therapeutic stability of polyherbal products (Fig. 1) [26].

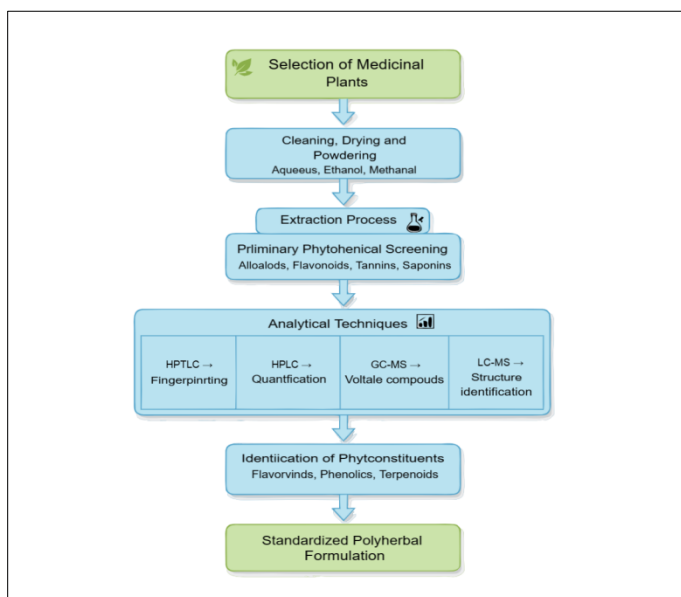


Figure 1: Schematic Representation of Phytochemical Profiling of Polyherbal Formulations [28]

## 5. Standardization of Polyherbal Formulations

The standardization of polyherbal formulations is one of the key conditions of quality, safety, and therapeutic consistency of a particular preparation. Because of the unpredictable and complicated nature of herbal constituents, variations in the source of plants, the condition of harvesting, the way it is processed and the storage may have a great impact on the end product. Thus, clear standardization procedures are necessary to obtain reproducible pharmacological results and be accepted by the regulation. Standardization combines the ancient knowledge with the current analytic methods to make sure that polyherbal formulations are able to reach the prescribed quality standards [29].

### 5.1 Need for Standardization

Standardization is mainly aimed at providing consistency in batches to batches and this is an important requirement in order to provide uniform therapeutic effectiveness. Phytochemical variability may cause changes in the pharmacological activity, which may influence clinical reliability. The use of standardization assists in the detection and management of such variations by set quality parameters [30].

The other important factor is to provide safety and effectiveness. Adequately standardized products reduce chances of contamination, adulteration and toxic constituents. It also makes sure that the formulation has the best concentrations of active compounds that are required to have any therapeutic effect. Without standardization, clinical application of polyherbal preparations can cause unforeseen results and lack of confidence in the patient [31].

### 5.2 Parameters for Standardization

Polyherbal formulations are standardized by using a set of organoleptic, physicochemical and microbiological analyses. The physical characteristics that are evaluated in organoleptic assessment are color, odor, taste, and texture. These parameters will give the initial signal of quality and it will assist in identifying any gross adulteration or degradation [32].

Physicochemical parameters are very important to determine purity and composition of the formulation. They are moisture content, ash values (total ash, acid insoluble ash), extractive values, pH and solubility. These parameters play a significant role in ensuring that the product is consistent and stable. Microbial limits testing will be used to make sure that the formulation does not contain some harmful microorganisms. Testing of overall bacterial count, fungal count and the absence of pathogenic microorganisms like Salmonella and Escherichia coli is essential to guarantee safety particularly in long term formulations [33].

### 5.3 Chromatographic Fingerprinting

Standardization of polyherbal formulations Chromatographic methods is important in offering a characteristic fingerprint profile of the chemical components in standardization of polyherbal preparations.

High-performance thin-layer chromatography (HPTLC) is common in creating fingerprint profiles that are signs of the existence of two or more phytochemicals in a formulation. These profiles are used as reference standards of quality control and also aid in the detection of adulteration or substitution. The process of marker-based standardization entails the discovery and the measurement of particular bioactive compounds, which are the cause of therapeutic effect. HPLC and LC-MS are the most common methods used to achieve this objective. Having accepted concentrations of marker compounds, one can achieve uniformity, potency and regulatory control of polyherbal preparations [34].

In general, standardization will fill the distance between the traditional herbal use and the modern pharmaceutical needs and improve the effectiveness and acceptance of polyherbal drugs globally [35].

## 6. Pharmacological Validation

The validation of therapeutic efficacy and scientific plausibility of polyherbal formulations is always necessary to

prove their pharmacological status. It includes systematic testing based on well-known experimental models in order to affirm anti-inflammatory and analgesic actions [36]. These investigations present mechanical evidence of the polyherbal preparations in the modulation of biological pathways that relate to inflammation and pain. *In vitro* and *in vivo* models are extensively used to determine their pharmacological potential so as to have the complete picture of their effects.

### 6.1 Anti-inflammatory Activity

Inflammation is a complex biological response involving multiple mediators such as prostaglandins, cytokines, nitric oxide, and reactive oxygen species. Polyherbal formulations exert anti-inflammatory effects by targeting these mediators through diverse mechanisms [37].

#### *In vitro* models

*In vitro* research gives some initial data on the anti-inflammatory activity on the basis of comparing the polyherbal extracts with particular molecular target. The cyclooxygenase (COX) inhibition assay is one of the most widely used assays and it is used to determine the potential of the formulation to inhibit the COX enzymes that produce prostaglandin, which is one of the major causes of inflammation. COX pathways inhibitors suggest the possibility of decreasing pain and inflammation [38].

The other model is the nitric oxide (NO) inhibition assay that determines the inhibition of nitric oxide generation in activated macrophages. Overabundance of nitric oxide is majorly involved in the mechanism of inflammation and inhibition of the same is an indication of the anti-inflammatory effects of the formulation [39].

#### *In vivo* models

*In vivo* models present a more detailed and physiologically applicable information about the anti-inflammatory activity. The paw swelling model due to carrageenan is one of the most popular acute inflammation models, in which lessening of paw swelling shows a successful anti-inflammatory effect. This model comes in handy especially when it comes to determining the inhibition of the prostaglandin-mediated inflammation [40].

Cotton pellet granuloma model is used to measure chronic inflammation and response of proliferative stage. It also assesses the development of granulomatous tissue, and a decrease in the weight of granuloma is an indicator of the capacity of the formulation in suppressing inflammatory mechanisms of chronic tissue growth [41].

### 6.2 Analgesic Activity

Analgesic activity of polyherbal formulations is evaluated using models that assess both central and peripheral mechanisms of pain modulation.

#### Central analgesic models

The central analgesic activity is an activity of modulating the perception of pain at the central nervous system level. Hot plate test This is most frequently employed to examine the effect that a thermal stimulus has on the response, where shortening of reaction time signifies analgesic effect mediated *via* central mechanisms. On the same note, tail flick test determines the time taken to respond to a heat stimulus placed on the tail. High latency indicates the presence of spinal reflexes and central pain inhibitory mechanisms, which means that they have great central analgesic effects [42].

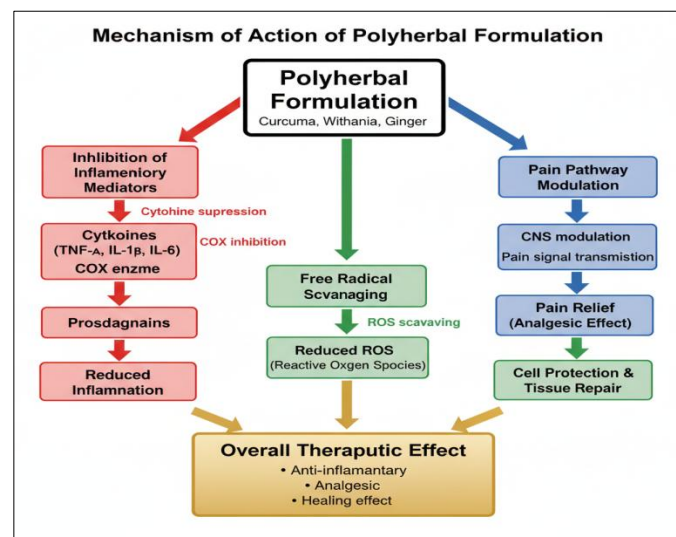


Figure 2: Mechanism of Anti-inflammatory and Analgesic Action of Polyherbal Formulations [45]

Table 3: Pharmacological Studies of Polyherbal Formulations [44]

S. No.	Experimental Model	Type of Study	Formulation Used	Dose	Activity Observed	Key Outcome
1	COX inhibition assay	<i>In vitro</i>	Polyherbal extract A	50–200 µg/mL	Anti-inflammatory	Significant COX inhibition
2	NO inhibition assay	<i>In vitro</i>	Polyherbal extract B	25–100 µg/mL	Anti-inflammatory	Reduced NO production
3	Carrageenan paw edema	<i>In vivo</i>	Polyherbal extract C	200 mg/kg	Anti-inflammatory	Reduced paw swelling
4	Cotton pellet granuloma	<i>In vivo</i>	Polyherbal extract D	300 mg/kg	Anti-inflammatory	Decreased granuloma weight
5	Hot plate test	<i>In vivo</i>	Polyherbal extract E	100 mg/kg	Central analgesic	Increased reaction time
6	Tail flick test	<i>In vivo</i>	Polyherbal extract F	150 mg/kg	Central analgesic	Increased latency
7	Acetic acid writhing	<i>In vivo</i>	Polyherbal extract G	200 mg/kg	Peripheral analgesic	Reduced writhing response
8	COX-2 selective assay	<i>In vitro</i>	Polyherbal extract H	50–150 µg/mL	Anti-inflammatory	Selective COX-2 inhibition
9	Lipoxygenase inhibition	<i>In vitro</i>	Polyherbal extract I	75–200 µg/mL	Anti-inflammatory	Reduced leukotriene synthesis
10	Formalin test	<i>In vivo</i>	Polyherbal extract J	250 mg/kg	Analgesic	Reduced pain phases
11	Xylene-induced ear edema	<i>In vivo</i>	Polyherbal extract K	200 mg/kg	Anti-inflammatory	Reduced ear swelling
12	Randall–Selitto test	<i>In vivo</i>	Polyherbal extract L	150 mg/kg	Analgesic	Increased pain threshold

## Peripheral analgesic models

Peripheral analgesic effect has been linked to the suppression of pain at the injury/inflamed place. Writhing test is a popular model of peripheral analgesic activity that is generated by acetic acid. It entails eliciting abdominal constrictions (writhes) in experimental animals and a decrease in the number of writhes is evidence of successful inhibition of peripheral pain mediators like prostaglandins [43].

## 7. Mechanisms of Action

The polyherbal formulations provide therapeutic efficacy in terms of inflammation and pain management due to their capacity to regulate several biochemical and molecular pathways at the same time. Polyherbal systems, as opposed to single-compound drugs, have a wide variety of bioactive constituents, which work in concert to produce greater pharmacological effects. Their anti-inflammatory and analgesic effects are related to the mechanisms of blocking inflammatory mediators, suppressing oxidative stress and synergistic effects of phytoconstituents (Fig. 2) [46].

### Cytokine Inhibition

Cytokines are the key players in the causes and development of inflammation. The amplification of the inflammatory reactions is caused by pro-inflammatory cytokines (tumor necrosis factor- $\alpha$ , TNF- $\alpha$ , interleukins, IL-1, IL-6, and so on). Polyherbal preparations have also been reported to inhibit the synthesis and release of these cytokines and hence lessen the inflammation on molecular level. Several phytochemicals, especially flavonoids and alkaloids, act on signaling pathways that include NF- $\kappa$ B and MAPK which are key factors controlling cytokine expression. This inhibition is useful in the control of acute and chronic inflammatory diseases [47].

### Prostaglandin Inhibition

The synthesis of the prostaglandins is a major mediator of the inflammation and pain process, which is mainly produced *via* the pathway of the cyclooxygenase (COX). Polyherbal preparations act as anti-inflammatory and analgesic *via* inhibiting the COX enzymes, particularly COX-2, which inhibits the production of prostaglandins. This action resembles that of NSAIDs except that the mechanism is associated with relatively lower side effects because of the existence of various bioactive compounds, which counteracts the overall effect. The low levels of prostaglandins result in the low levels of pain perception, swelling, and inflammation [48].

### Free Radical Scavenging

One of the significant causes of pathogenesis of tissue injuries and inflammation is oxidative stress. Overproduction of reactive oxygen species (ROS) may cause cellular damage, slowdown of tissue repair, and aggravation of the inflammatory reactions. Polyherbal extracts highly

concentrated in phenolic compounds and flavonoids have a high antioxidant activity that inhibits the formation of free radical and reinforces endogenous antioxidant systems. The activity aids in preserving the integrity of the cell, diminishes the oxidative stress, and enhances faster recovery [49].

## Synergistic Interactions

The synergistic mode of action of polyherbal formulations is one of the most important. Multiple plant constituents can interact in a complementary and additive fashion with one constituent potentially improving the activity, stability or bioavailability of another. As an example, some of the compounds can enhance the absorption of the active phytochemicals and some can reduce the possible toxicity. Using this synergism allows formulating the product that simultaneously targets various pathways involved in inflammation and pain and yield better therapeutic efficacy and fewer adverse effects [50].

## 8. Safety and Toxicological Evaluation

The safety and toxicological analysis are an important aspect in the development and clinical acceptance of polyherbal formulations. The use of herbal medicines might be thought of as relatively safe since its natural source implies that its use is safe, but scientific validation is required to confirm this, particularly in long-term use. Thorough toxicological research is useful in establishing safe dose levels, establishing possible side effects, and the therapeutic window of polyherbal preparations [51].

### Acute Toxicity

Acute toxicity research is aimed at determining the short-term negative consequences of a single dose or numerous administrations of a drug in a specific duration of time (24 hours). The studies are usually conducted in experimental animal models with guidelines that are standard like in OECD. Mortality, behavioral changes, physical evidence of toxicity, biochemical changes are the parameters that are monitored carefully. The margin of safety is usually large in polyherbal preparations, and numerous studies have shown that there was no significant toxicity at any dosage level of the therapy. The calculation of the median lethal dose (LD<sub>50</sub>) is useful because it gives vital data on the safety profile and assists in the choice of dose to use in subsequent pharmacological research [52].

### Chronic Toxicity

Chronic toxicity studies determine the effects of a continued administration of polyherbal preparations over a period, and this period is usually weeks to months. These are critical studies in measures of long-term safety, particularly formulations that are supposed to be used in chronic inflammatory and pain diseases. Body weight, food intake, hematological indices, biochemical markers, organ functions, as well as histopathology of vital organs are examined as parameters. A majority of polyherbal preparations have

minimal or no toxicity in the course of prolonged administration when used within the recommended dosages. Nonetheless, the phytochemical variation and lack of proper standardization can affect safety, and it is necessary to carefully evaluate them [53].

### Herbal Safety vs Synthetic Drugs

Polyherbal preparations have a better safety profile than synthetic drugs like NSAIDs and opioids. Drugs which are synthetic though effective are usually linked to side effects, such as gastro-intestinal irritations, kidney damages, cardiovascular complications, tolerance, and dependency. Conversely, polyherbal preparations are less likely to have side effects since they are well balanced and have synergies among phytoconstituents [54].

Moreover, some herbal constituents can have protective properties against toxicity, e.g. antioxidant action leading to the minimization of oxidative damage. It should be noted though that natural does not necessarily mean safe. The problems of contamination, adulteration, herb-drug interactions and the variation of the raw materials should be taken seriously by ensuring that there is proper standardization and quality control [55].

### 9. Challenges and Limitations

Despite the promising therapeutic potential of polyherbal formulations in the management of inflammatory and pain-related disorders, several challenges and limitations hinder their widespread acceptance and integration into modern healthcare systems. Addressing these issues is essential to ensure their scientific credibility, regulatory approval, and clinical reliability [56].

#### Lack of Standardization

The lack of clearly defined standardization procedures is one of the biggest problems that can be related to polyherbal formulations. With multi-herb combinations, it is hard to standardize the composition, quality, and potency since they are complex. The differences in the extraction process, in formulation procedures, and the lack of verified marker substances usually result in the discrepancies in the therapy results. In the absence of appropriate standardization, reproducibility of results and regulatory approval is a significant issue [57].

#### Variability in Plant Sources

Medicinal plants may have a wide difference in phytochemical composition with regard to geographical, climatic, soil quality, harvesting time, and post-harvest processing. This inconsistency has a direct impact on the concentration of bioactive constituents in polyherbal preparations, which results in inconsistency in efficacy and safety. Also, challenges like adulteration, replacement and heavy metal or pesticide contamination also complicate the quality assurance of herbal

products. The challenge of using original and quality raw materials is therefore one of the crucial ones [58].

### Limited Clinical Trials

Despite the fact that many *in vitro* and *in vivo* experiments confirm the anti-inflammatory and analgesic effects of polyherbal preparations, there are no well-designed clinical trials to confirm the effectiveness and safety of these preparations in humans. The majority of the existing research is preclinical with little information regarding the safety in the long-term, optimal dosing, and therapeutic responses in various patients. This lacks strong clinical evidence that would support their use in evidence-based medicine and restricts the inclusion of those in the conventional treatment guidelines [59].

### 10. Conclusion

Polyherbal formulations represent a promising and holistic therapeutic approach for the management of inflammatory and pain-related disorders. Their significance lies in the presence of diverse bioactive phytoconstituents that act through multiple mechanisms, including modulation of inflammatory mediators, inhibition of oxidative stress, and regulation of pain pathways. The concept of synergism further enhances their therapeutic efficacy, allowing improved outcomes with reduced toxicity compared to conventional single-drug therapies.

This review highlights the critical importance of standardization, phytochemical profiling, and pharmacological validation in ensuring the quality, safety, and reproducibility of polyherbal formulations. Advances in analytical techniques and experimental models have provided substantial evidence supporting their anti-inflammatory and analgesic potential. Moreover, their comparatively favorable safety profile makes them attractive alternatives to synthetic drugs, which are often associated with adverse effects during long-term use. Despite these advantages, the transition of polyherbal formulations from traditional use to mainstream clinical application requires rigorous scientific validation. The lack of standardized protocols and limited clinical evidence remain significant barriers to their widespread acceptance in evidence-based medicine.

In conclusion, polyherbal formulations hold strong potential as effective, safe, and multi-targeted therapeutic agents. However, well-designed clinical trials, standardized manufacturing processes, and integration of modern technologies are essential to fully establish their therapeutic value and ensure their successful incorporation into modern healthcare systems.

**ACKNOWLEDGEMENT:** Author are thankful to his supervisor and co-supervisor for their valuable guidance throughout this study.

**CONFLICT OF INTEREST:** Nil

## REFERENCES:

- Ji J, Yuan M, Ji RR. Inflammation and pain. In *Neuroimmune interactions in pain: mechanisms and therapeutics* 2023 May 6 (pp. 17-41). Cham: Springer International Publishing.
- Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nature Reviews Cardiology*. 2018 Sep;15(9):505-22.
- Kasturi J, Palla PR, Bakshi V, Bogg N. Non-steroidal anti-inflammatory drugs: an overview. *Journal of Drug Delivery & Therapeutics*. 2019 Jan 2.
- Al-Worafi YM. Evidence-Based Complementary, Alternative, and Integrated Medicine and Efficacy and Safety: Concepts. In *Handbook of Complementary, Alternative, and Integrative Medicine* 1-13. CRC Press.
- Sivaji S, Thangavelu S, Seetharaman B, Jayaraman L, Kaur J, Dhar SK, Mudgal G, Vasantharekha R. Synergistic Effects of Multi-Herbal and Herb-Drug Combinations and Their Molecular Mechanisms of Action and Clinical Relevance. In *Exploring Herbal Synergies for Optimal Human Health* 2026 (pp. 385-410). CRC Press.
- Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, Pareek A, Chaturgoon AA. Moringa oleifera: an updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. *International journal of molecular sciences*. 2023 Jan 20;24(3):2098.
- Bommakanti V, Puthenparambil Ajikumar A, Sivi CM, Prakash G, Mundanat AS, Ahmad F, Haque S, Prieto MA, Rana SS. An overview of herbal nutraceuticals, their extraction, formulation, therapeutic effects and potential toxicity. *Separations*. 2023 Mar 6;10(3):177.
- Shruti SS, Sharma P, Dhaked P, Sharma R, Parmar A. Pharmacognostic Standardization and Quality Controlled Parameters of Polyherbal Formulations. *International Journal of Pharmacognosy and Herbal Drug Technology*. 2025 Oct 29:08-22.
- Kumar MM, Sharma A, Rinka Juneja DT, Arif MM, Singh MA, Kumar A. Biological evaluation of herbal preparations and formulation standardization: a literature study. *J Neonatal Surg*. 2025;14(8s):273.
- Gogoi D, Kiewhuo K, Chutia H, Vaikundamani S, Sastry GN, Mahanta HJ. A systemic investigation of the phytochemical space in polyherbal formulations of India: A curated database. *Computers in Biology and Medicine*. 2025 Sep 1;196:110760.
- Bansod MS, Kagathara VG, Somkuwar AD. Evaluation of analgesics and anti-inflammatory activity of a poly-herbal formulation. *Int J PharmTech Res*. 2010;2(2):1520-7.
- Collares LJ, Turchen LM, Guedes RN. Research trends, biases, and gaps in phytochemicals as insecticides: literature survey and meta-analysis. *Plants*. 2023 Jan 10;12(2):318.
- Rasoanaivo P, Wright CW, Willcox ML, Gilbert B. Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. *Malaria journal*. 2011;10(Suppl 1):S4.
- Mandal S. Murraya koenigii: A Source of Bioactive Compounds for Inflammation and Pain Management. *Current Bioactive Compounds*. 2025:E15734072348822.
- Alamgir AN. Biotechnology, in vitro production of natural bioactive compounds, herbal preparation, and disease management (treatment and prevention). In *Therapeutic use of medicinal plants and their extracts: volume 2: phytochemistry and bioactive compounds* 2018 Jun 24 (pp. 585-664). Cham: Springer International Publishing.
- Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, Dubey SP, Khan N, Jain DK. Polyherbal formulation concept for synergic action: A review. *Journal of Drug Delivery & Therapeutics*. 2019 Jan 2.
- Verma S, Panchpuri M, Dwibedi V, Rath SK, Devi A. Exploring herbal synergies in the management of rheumatoid arthritis: a multifaceted approach to inflammation and joint health. *Inflammopharmacology*. 2025 Nov;33(11):6457-84.
- Thabrew MI, Dharmasiri MG, Senaratne L. Anti-inflammatory and analgesic activity in the polyherbal formulation Maharasnadhi Quathar. *Journal of ethnopharmacology*. 2003 Apr 1;85(2-3):261-7.
- Rajani M, Kanaki NS. Phytochemical standardization of herbal drugs and polyherbal formulations. In *Bioactive molecules and medicinal plants* 2008 (pp. 349-369). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Nagori M, Rajput D, Choudhary G, Khabiya R. Qualitative and quantitative methods of phytochemical analysis. *Pharmacognosy and Phytochemistry: Principles, Techniques, and Clinical Applications*. 2025:143-66.
- Hardiningsih DT, Hidayat AT, Delfira A. Qualitative identification of alkaloid compounds in Moringa oleifera leaf extract. *Proceedings Academic Universitas Jambi*. 2025 Nov 27;1(2):468-75.
- Rahman MM, Islam MR, Shohag S, Hossain ME, Rahaman MS, Islam F, Ahmed M, Mitra S, Khandaker MU, Idris AM, Chidambaram K. The multifunctional role of herbal products in the management of diabetes and obesity: a comprehensive review. *Molecules*. 2022;27(5):1713.
- Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK, Devi R. Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in pharmacology*. 2022 Feb 14;13:806470.
- Sahare AY, Akare SC, Agrawal RB, Gotephode SN, Gajbhiye SA. Standardization and Quality Control of Ayurvedic Polyherbal Formulations. *Int Jou of Pharmacog and Herbal Drug Technology*. 2025:103-20.
- McDonald JG, Matthew S, Auchus RJ. Steroid profiling by gas chromatography-mass spectrometry and high performance liquid chromatography-mass spectrometry for adrenal diseases. *Hormones and Cancer*. 2011;2(6):324-32.
- Lalchandani DS, Chenkual L, Porwal PK. Stability of herbal formulations: emerging trends and techniques. *Formulating Pharma-, Nutra-, and Cosmeceutical Products from Herbal Substances: Dosage Forms and Delivery Systems*. 2025 Aug 26:195-217.
- Sulaiman CT, Anju K, Anandan EM, Balachandran I. Synergistic interactions of phytochemicals in polyherbal formulation enhance the chemical transformations of active constituents. *Journal of Applied Spectroscopy*. 2021 Mar;88(1):181-6.
- Dinakaran SK, Chelle S, Avasarala H. Profiling and determination of phenolic compounds in poly herbal formulations and their comparative evaluation. *Journal of traditional and complementary medicine*. 2019 Oct 1;9(4):319-27.
- Awasthi H, Mani D, Nath R, Nischal A, Usman K, Khattri S. Standardization, preparation and evaluation of an Ayurvedic polyherbal formulation in a capsule dosage form

- suitable for use in clinical trials. *Indo Am J Pharm Res.* 2014;4(10):4093-9.
30. Dai X, Mei Y, Cai D, Han W. Standardizing CAR-T therapy: Getting it scaled up. *Biotechnology advances.* 2019;37(1):239-45.
  31. Salgueiro L, Martins AP, Correia H. Raw materials: the importance of quality and safety. A review. *Flavour and Fragrance Journal.* 2010 Sep;25(5):253-71.
  32. Monika T, Ilavarasi L, Abinaya T, Saravanadevi MD, Rani RK, Meenakumari R. Standardization of A classical siddha poly herbal formulation "Nannari Mathirai" through organoleptic character, physiochemical and phytochemical analysis. *Phytochem. Ana. Eur. J. Phar. Med. Res.* 2020;7.
  33. Bhardwaj AK, Kashyap K, Hait M, Bera SK, Dewangan H. Physicochemical Characterization of Rhizome of *Curcuma caesia* Roxb. *ES Food and Agroforestry.* 2023;11(7):813.
  34. Kumar B, Sharma S, Singh SP, Adhikari D, Kumar S, Srivastava S. Ecological niche modelling guided chemotypic analysis of *Ageratum conyzoides* L. from varied geography of India. *South African Journal of Botany.* 2024 May 1;168:372-83.
  35. Hossain CM, Gera ME, Ali KA. Current status and challenges of herbal drug development and regulatory aspect: a global perspective. *AJPCR.* 2022;15(2):31-41.
  36. Dubey S, Dixit AK. Preclinical evidence of polyherbal formulations on wound healing: A systematic review on research trends and perspectives. *Journal of Ayurveda and integrative Medicine.* 2023 Mar 1;14(2):100688.
  37. Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezme MN. The crucial roles of inflammatory mediators in inflammation: A review. *Veterinary world.* 2018 May 15;11(5):627.
  38. Rao P, Knaus EE. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. *Journal of pharmacy & pharmaceutical sciences.* 2008 Sep 20;11(2):81s-110s.
  39. Król W, Czuba ZP, Threadgill MD, Cunningham BD, Pietsz G. Inhibition of nitric oxide (NO•) production in murine macrophages by flavones. *Biochemical Pharmacology.* 1995 Sep 28;50(7):1031-5.
  40. Martínez-Rizo AB, Fosado-Rodríguez R, Torres-Romero JC, Lara-Riegos JC, Ramírez-Camacho MA, Herrera AL, de la Torre FE, Góngora EC, Arana-Argáez VE. Models *in vivo* and *in vitro* for the study of acute and chronic inflammatory activity: A comprehensive review. *International immunopharmacology.* 2024 135:112292.
  41. Afsar SK, Kumar KR, Gopal JV, Raveesha P. Assessment of anti-inflammatory activity of *Artemisia vulgaris* leaves by cotton pellet granuloma method in Wistar albino rats. *Journal of pharmacy research.* 2013;7(6):463-7.
  42. Martucci KT, Mackey SC. Neuroimaging of pain: human evidence and clinical relevance of central nervous system processes & modulation. *Anesthesi.* 2018;128(6):1241.
  43. Sharma S, Khare S, Dubey BK, Joshi A, Jain A. Analgesic activity of poly herbal formulation in experimental rats by acetic acid induced writhing test model and Hot plate model. *Journal of Drug Delivery & Therapeutics.* 2019;9.
  44. Aladejana EB. Biological Properties of Polyherbal Formulations: A Review of their Antimicrobial, Anti-inflammatory, Antioxidant, and Toxicological Activities. *Pharmacognosy Journal.* 2023 Sep 1;15(5).
  45. Meera S, Kumar NS, Guptatyam VS. Screening of anti-arthritis, anti-inflammatory and analgesic activity of a polyherbal formulation. *International Journal of Pharmacology.* 2008 Aug 15;4(5):398-402.
  46. Balkrishna A, Ranjan R, Sakat SS, Sharma VK, Shukla R, Joshi K, Devkar R, Sharma N, Saklani S, Pathak P, Kumari P. Evaluation of polyherbal ayurvedic formulation 'Peedantak Vati' for anti-inflammatory and analgesic properties. *Journal of ethnopharmacology.* 2019 May 10;235:361-74.
  47. Singh AK, Kumar P, Rajput VD, Mishra SK, Tiwari KN, Singh AK, Minkina T, Pandey AK. Phytochemicals, antioxidant, anti-inflammatory studies, and identification of bioactive compounds using GC-MS of ethanolic novel polyherbal extract. *Applied Biochemistry and Biotechnology.* 2023 Jul;195(7):4447-68.
  48. Mukhopadhyay N, Shukla A, Makhla PN, Kaki VR. Natural product-driven dual COX-LOX inhibitors: Overview of recent studies on the development of novel anti-inflammatory agents. *Heliyon.* 2023 Mar 1;9(3).
  49. Chatterjee S. Oxidative stress, inflammation, and disease. *In Oxidative stress and biomaterials 2016;* 35-58.
  50. Jayaraman L, Lakshitha D, Sivaji S, Vasantharekha R, Seetharaman B, Mudgal G. Future Directions in Herbal Synergy Research: Emerging Trends and Prospective Areas. *Exp. Herb. Synerg. for Opt. Human Health.*:411-35.
  51. Banerjee P, Maity S, Das T, Mazumder S. A double-blind randomized placebo-controlled clinical study to evaluate the efficacy and safety of a polyherbal formulation in geriatric age group: A phase IV clinical report. *Journal of ethnopharmacology.* 2011 Mar 24;134(2):429-33.
  52. Saganuwan SA. Toxicity studies of drugs and chemicals in animals: an overview. *Bulgarian Journal of Veterinary Medicine.* 2017 Dec 1;20(4).
  53. Autade KA, Pandhare RB. Toxicity Profiling of a Polyherbal formulation for hepatic health: acute and subacute evaluation. *Toxi. Res.* 2025;14(2):tfaf040.
  54. Bhat AH, Handa K. Anti-Inflammatory and Pain-Relieving Herbs: Combinations That Reduce Inflammation and Alleviate Pain. *In Exploring Herbal Synergies for Optimal Human Health* (pp. 178-202). CRC Press.
  55. Singh D, Cho WC, Upadhyay G. Drug-induced liver toxicity and prevention by herbal antioxidants: an overview. *Frontiers in physiology.* 2016;6:363.
  56. Rajalekshmi R, Agrawal DK. Therapeutic efficacy of medicinal plants with allopathic medicine in musculoskeletal diseases. *International journal of plant, animal and environmental sciences.* 2024;14(4):104.
  57. Khajuria RK, Agarwal SG. Principles of Quality Control, Standardization and Chemo profiling of Medicinal Plants and ISM Preparations. *Approaches towards Evaluation of Medicinal Plants prior to Clinical Trials.* 2006 Nov 8:89.
  58. Amrutanand ST, Annegowda HV, Das K. Influence of pre and post-harvest technologies in effective yield, yield attributes and quality enhancement of medicinal and aromatic plants for healthy life. *Ann. Phytomed.* 2021;10(1):45-61.

**How to Cite this article:**

Abhay Kumar Mourya AK, Makhija M, Mishra SB, Gupta M. Standardization, phytochemical profiling, and pharmacological validation of polyherbal formulations for auto-inflammatory and analgesic applications. *International Journal of Pharmaceutical Science and Medicine* 2025; 3(3): 85-93.