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Biological Evaluation Of Herbal Preparations And Formulation Standardization: A Literature Study

Mr. Mohit Kumar[1], Dr. Amit Sharma[*2], Rinka Juneja[3], Dr. Tarun Parashar[4], Mr. Mohammad Arif[5], Mr. Aniruddh Dev Singh[6], Dr. Ashok Kumar[7]

[1]Assistant Professor, Teerthankar Mahaveer college of Pharmacy, Teerthankar Mahaveer university, Moradabad, Uttar Pradesh, India

[*2]Professor Professor ,HOD Sai School of pharmaceutical Education and research/PCI-3987) Sri Sai University Palampur Himachal

[3]Assistant professor, School of Pharmaceutical Science , CT University,, Ludhiana, 142024

[4] Associate Professor, School of Pharmacy & Research, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand 248007

[5]Associate Professor, MITCOP, MIT Campus, Ram Ganga Vihar Phase 2, Moradabad, Uttar Pradesh, India

[6]Assistant Professor, Teerthankar Mahaveer college of Pharmacy , Teerthankar Mahaveer university2, Moradabad, Uttar Pradesh, India

[7]Head of Department, School of Pharmaceutical Sciences, Jigyasa University, Dehradun, Uttarakhand-248197, India

[*]**Corresponding author:**

Email ID: mohitgoyal21111@gmail.com

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ABSTRACT

The use of herbal medicines has gained significant attention in recent years due to their potential therapeutic benefits and minimal side effects. However, the lack of standardization in herbal preparation and formulation has raised concerns about their safety and efficacy. This literature study aims to evaluate the biological activity of various herbal preparations and highlight the importance of formulation standardization. The study extensively reviews the literature on the biological evaluation of herbal preparations, including the assessment of phytochemical composition, antioxidant activity, anti-inflammatory properties, and cytotoxicity. Additionally, the study discusses the current challenges in standardizing herbal formulations, including the variability in plant material, extraction methods, and manufacturing processes. The study concludes that the biological activity of herbal preparations depends on several factors, including the selection of plant material, preparation method, and formulation standardization. Therefore, standardized protocols for the preparation and formulation of herbal medicines are essential to ensure their safety, efficacy, and reproducibility.

Keywords: *Herbal medicines, Standardization, Biological activity, Therapeutic benefits, Toxicity, Regulatory compliance*

1. INTRODUCTION

Herbal formulations are becoming increasingly popular due to their potential health benefits and lower risk of side effects compared to synthetic drugs. However, standardizing herbal formulations can be challenging due to their chemical constituents and the inconsistency of the active constituents in different plant species and even within the same species. Unlike synthetic drugs, herbal formulations contain a mixture of active and inactive constituents, which can fluctuate significantly liable on the geographic location, and growing conditions. Moreover, the active constituents in different parts of the same plant can also vary, making it difficult to ensure consistency and quality across different batches of herbal formulations.

For generations, people have employed herbal remedies as a source of remedies for various disorders, and their popularity has only increased in recent years. They have grown significant courtesy recently due to their potential therapeutic benefits and minimal side effects. Many people choose to use herbal medicines as a complementary or alternative treatment option

to conventional medicine. The biological evaluation of herbal preparations involves assessing the effects of herbal extracts or preparations on living organisms, such as animals or humans. This evaluation is done to regulate the safety, efficacy, and an approach of action of herbal products. There are several ways to evaluate the biological effects of herbal preparations such as Toxicity testing, Pharmacological screening, Bioavailability testing, and Clinical trials. However, there is a lack of standardization in herbal preparation and formulation, which has raised concerns about their safety and efficacy.

The completed products should preferably be standardized, which means that they should be adjusted to a predetermined content of elements with established therapeutic action as far as this is practicable, due to the batch-to-batch variability inherent in biological material. In addition to quality control methods based on chemical markers, pattern-oriented methods based on fingerprints are emerging tools that are thought to be very helpful for the quality control of herbal products because they are thought to be better able to depict the intricate nature of phytochemical preparations than the more traditional marker-based methods. There are numerous ways to guarantee proper beginning material quality and manufacturing quality at all stages. The proper strategy must be chosen individually for each activity based on the task at hand as well as the equipment that is accessible. Numerous pharmacopoeia monographs provide macroscopic, microscopic, and TLC-based procedures for identification as well as HPLC- and occasionally GC-based methods for quantitative measurements. Although these techniques are reliable, fast, and inexpensive, they won't be adequate for all applications [1]

The practise of prescribing a set of standards or innate qualities, constant parameters, and unmistakable qualitative and quantitative values that convey an assurance of quality, efficacy, safety, and repeatability is known as standardisation of herbal medicines. Creating and approving technical standards is the procedure at hand. Experimentation and observation are used to develop precise criteria, and this process eventually results in the prescription of a particular set of traits displayed by a given pharmaceutical. Standardisation is a tool in the quality assurance process as a result. One of the main reasons for the lack of standardization is the natural chemical variability of herbs, which can be affected by factors such as growing conditions, harvesting methods, and processing techniques. This variability makes it difficult to ensure that each batch of an herbal product contains a consistent amount of active ingredients, which can affect its safety and efficacy. Another factor contributing to the lack of standardization is the absence of a regulatory framework for herbal medicines in many countries. Contrary to prescription pharmaceuticals, herbal remedies are frequently marketed as dietary supplements and are not subject to the same stringent laws and quality assurance procedures. The necessity for standardization of these herbal drugs has undoubtedly increased with the rise in the usage of herbal medicines and worries about their safety and efficacy. Most nations use the WHO-set standards for these medications as a reference point [2]since they are internationally recognized. To identify, authenticate, and better understand a substance's chemical makeup, standardization comprises both internal and exterior examinations, ash values, extractive values, and a number of other characteristics.

The lack of standardization in herbal preparation and formulation can have several consequences. For instance, it can lead to variations in the effectiveness of herbal products, making it difficult to predict how well they will work for a given condition. It can also increase the risk of adverse effects, as different batches of the same product may contain varying amounts of potentially toxic compounds. In addition, the lack of standardization can make it challenging for healthcare providers to determine the appropriate dosage of an herbal product. This can be especially problematic when patients are taking multiple herbal products simultaneously or when they are taking prescription medications that may interact with the herbal products. The necessity for standardisation of these herbal drugs has undoubtedly increased with the rise in the usage of herbal medicines and worries about their safety and efficacy. Most nations use the WHO-set standards for these medications as a reference point [2] since they are internationally recognised. To identify, authenticate, and better understand a substance's chemical makeup, standardisation comprises both internal and exterior examinations, ash values, extractive values, and a number of other characteristics.

1.1 Need for The Literature Study

A literature study on the biological evaluation of herbal preparations and formulation standardization can provide valuable insights into the scientific evidence for their therapeutic potential.

- Traditional knowledge validation: Traditional medical systems have utilized a variety of herbal treatments for millennia, but there is often a lack of scientific evidence to support their use. A review can help validate traditional knowledge by identifying the scientific evidence for the therapeutic effects of herbal preparations.
- Safety assessment: A review can provide a comprehensive assessment of the safety profile of herbal preparations, including potential toxicity and adverse effects. This information can be used to establish guidelines for the safe use of herbal preparations.
- Standardization: A review can help establish standardized methods for the preparation and characterization of herbal preparations, which is essential for ensuring consistent quality and efficacy.
- Identification of active compounds: Many herbal preparations contain complex mixtures of bioactive compounds, and a review can help identify the specific compounds responsible for their therapeutic effects. This information can be used to develop more targeted and effective herbal preparations.

- Novel drug discovery: Herbal preparations are a rich source of natural products, and a review can help identify novel compounds with potential for drug discovery. This can lead to the progress of new drugs grounded on organic substances.
- Regulatory compliance: The regulation of herbal products is often complex and varies widely between different countries and regions. A review can help identify the regulatory requirements for herbal products and establish guidelines for compliance.

1.2 Motivation for The Literature Study

The motivation for a literature study on the biological evaluation of herbal preparations and formulation standardization is to gain a better understanding of the scientific evidence for the "safety, efficacy, and quality", of herbal products. This literature study intends to assess the biological activity of various herbal preparations and highlight the importance of formulation standardization. The study extensively analyses the literature on the biological evaluation of herbal preparations, including the assessment of phytochemical composition, antioxidant activity, anti-inflammatory properties, and cytotoxicity. Additionally, the study discusses the current challenges in standardizing herbal formulations, including the variability in plant material, extraction methods, and manufacturing processes.

1.3 Challenges of The Literature Study

The literature study on the biological evaluation of herbal preparations and formulation standardization faces several challenges. Some of these challenges include:

- Lack of standardized methods: The lack of standardized methods for evaluating the biological activity of herbal preparations makes it difficult to compare results across different studies. This can lead to inconsistencies in the literature, making it difficult to draw firm conclusions.
- The complexity of herbal preparations: Herbal preparations often contain complex mixtures of compounds, making it difficult to identify the active constituents responsible for their biological activity. This can make it challenging to design experiments that accurately reflect the effects of the whole preparation.
- Variability in quality and composition: Depending on elements like the source of the plant material, the extraction process, and the formulation, the quality and composition of herbal remedies can vary greatly. This can make it difficult to replicate results across different studies, and may also affect the safety and efficacy of the preparations.
- Lack of standardization in the formulation: There is often a lack of standardization in the formulation of herbal preparations, which can lead to variability in the concentration and bioavailability of active constituents. Inferring significant conclusions regarding the biological activity of herbal medicines from the results of various research might be challenging as a result of this.
- Ethical considerations: There are ethical considerations involved in the study of herbal preparations, particularly in terms of the potential harm to humans and animals. This can make it challenging to design experiments that are both effective and ethical.
- Funding and resources: There may be limited funding and resources available for the study of herbal preparations, particularly in comparison to the resources available for the study of synthetic drugs. This can make it difficult to conduct large-scale studies that are necessary for establishing the safety and efficacy of herbal preparations.

1.4 Scope and Organization of The Survey

The scope of a literature survey on the biological evaluation of herbal preparations and formulation standardization would involve reviewing the existing literature related to the use of herbal preparations in healthcare, including studies on their biological activity, safety, and efficacy. This would include a review of the many techniques used to assess the biological activity of herbal preparations, as well as the challenges and limitations associated with these methods. The survey would also cover the different approaches to standardizing the formulation and manufacturing of herbal preparations, as well as the regulatory frameworks that govern their use. Finally, the survey would appraise the existing indication on the safety and efficacy of herbal preparations, including their use in clinical trials for specific health conditions.

2. BIOLOGICAL EVALUATION OF HERBAL PREPARATIONS

Herbal preparations have been used for centuries as traditional medicine and continue to be widely used today. However, there is often limited scientific evidence to support their efficacy and safety. Therefore, biological evaluation of herbal preparations is important to determine their potential benefits and risks. Biological evaluation of herbal preparations involves the investigation of their active constituents, pharmacological properties, toxicity, and potential interactions with other drugs. This process can include various laboratory and clinical tests to assess the effects of the herbal preparation on living

organisms.

Examine [3] the impact of an ethanolic extract of *Pupalia lappacea* (L.) Juss. (Amaranthaceae) leaves on adipocytes, blood glucose levels, and lipid levels in rats with diabetes caused by streptozotocin (STZ). On STZ-induced diabetic rats, the extract's antidiabetic and hypolipidemic efficacy was assessed in vivo. On the 3T3-L1 cell line, the antiadipogenic activity was assessed in vitro using simvastatin as the reference medication.

Examine [4] the impact of a *Glycyrrhiza glabra* ethanol extract on the HT-29 colon cancer cell line's HSP90 expression, proliferation, and apoptosis. 50, 100, 150, and 200 g/ml of the extract were used to treat HT-29 cells. They employed the MTT assay and, separately, flow cytometry techniques to assess cell growth and apoptosis. To assess the levels of HSP90 gene expression, RT-PCR was additionally performed.

Datura innoxia Mill.[5] was biologically assessed using a variety of assays, including cytotoxic, protein kinase inhibition, antioxidant, and antibacterial. A novel source of "natural antioxidants, antimicrobials, and anticancer chemicals" might be thought of as D. innoxia. "Total phenolic and flavonoid contents were determined colorimetrically and specific polyphenols", were quantified by HPLC-DAD analysis

Strobilanthes crispus [6] was examined for disparities in the secondary metabolites constituents as well as for their antioxidant and anticancer effects. Using UHPLC, phenolic acids and flavonoids were discovered. To assess the antioxidant activity, "1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP) assays", were utilized. "MTT assay", was used to assess the antitumor activity of extracts against the HeLa cancer cell line.

Investigated [7] the biological potential by examining the antibacterial, antioxidant, and antiproliferative activities. All preparations were potent against the fungus "*Saccharomyces cerevisiae* and *Aspergillus niger"*, nonetheless, only the vital oils of sweet basil and spearmint showed cytotoxicity against prevalent foodborne infections. Variable levels of antioxidant efficacy were discovered when antioxidants were measured using the "DPPH and ABTS radical scavenging activity assays". The "Sulforhodamine B (SRB) assay", was used to test and evaluate their antiproliferative capability in opposition to a group of human cancer cell lines.

The chemical makeup [8] of the vital oil extracted from *Piper xylosteoides* (Kunth) Steud leaves was examined, as well as its antibacterial and drug-enhancing capability against clinically significant bacterial and fungal strains. The broth microdilution and half-maximal inhibitory concentrations were applied in the antibacterial analysis. Subinhibitory amounts of the essential oil were mixed with the antifungal medication fluconazole, the antibiotics norfloxacin, gentamicin, and erythromycin to assess the drug boosting potential. By examining morphological change in wet chambers, the effect of the in vitro therapy on fungal pathogenicity was ascertained.

Determined [9] the amounts of, "l(+)-ascorbic acid (ASA), total phenolic (TPC), total flavonoid (TFC), total phenolic acid (TPAC), and total phenolic acid", in the white mulberry. The antioxidant potential was evaluated using the "FRAP (ferric-reducing/antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assays", while the AChE inhibitory activity was assessed using the Ellman assay for aqueous extracts.

Evaluated [10]the propolis from Poland's medicinal potential and established its botanical origin by "GC-MS analysis". The chemical constituents of *Populus nigra* bud exudates was identified, but glycerol esters of phenolic acids, extremely high concentrations of p-coumaric and ferulic acids, and their benzyl esters were also found. These elements are typical of *Populus tremula* buds. Additionally, they examined how propolis extracts affected nine human cancer cell lines' ability to proliferate. The dichloromethane extract was also shown to have modest antifungal efficacy and potential antibacterial activity.

Create [11] neem leaf crude extracts using a maceration method and various polarity organic solvents. Then, using spectroscopy, evaluated "phytochemical screening, total phenol content, and antioxidant activity". Traditional, "Foline-Ciocalteu reagent (FCR) and a, a-diphenyl-b-picrylhydrazyl (DPPH) techniques", were used to identify total phenol concentration and free radical scavenging activity.

Looked[12] for chemical ingredients and novel antibacterial agents in vital oils extracted from the tubers of "*Curcuma aeruginosa* (C. aeruginosa) Roxb., *Curcuma glans* K. Larsen & J. Mood, and *Curcuma cf. xanthorrhiza* Roxb". Hydro-distillation was used to extract the oils, which were then analyzed using gas chromatography/mass spectrometry. The antibacterial activity was investigated using "agar-well diffusion method, and the minimum inhibitory concentration (MIC)" against four bacterial strains and yeast was established using broth-micro dilution techniques.

Analyzed[13] the systemized vital oils extracted from *Polygonum hydropiper* to see if they have anticholinesterase and antioxidant properties. The samples' inhibitory potentials against "acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)" were assessed using Ellman's test. "1,1-diphenyl,2-picrylhydrazyl (DPPH), 2,2-azinobis[3-ethylbenzthiazoline]-6-sulfonic acid (ABTS), and hydrogen peroxide (H_2O_2) free radical scavenging assays", were used in the antioxidant experiments. Review of biological evaluation of herbal preparations are shown in the Table 1.

Table 1: Review of biological evaluation of herbal preparations

Citation No	Author	Year	Herbal plants assayed	Methods used for biological evaluation
[3]	Kumar *et al*	2016	*Pupalia lappacea*	invitro cytotoxicity
[4]	Nourazarian *et al*	2016	*Glycyrrhiza glabra*	MTT assay
[5]	Fatima *et al*	2016	*Datura innoxia* Mill	HPLC-DAD
[6]	Ghasemzadeh *et al*	2015	*Strobilanthes crispus*	UHPLC, DPPH, MTT
[7]	Fitsiou *et al*	2016	*"Ocimum basilicum, Mentha spicata, Pimpinella anisum* and *Fortunella margarita"*	"DPPH, ABTS radical scavenging activity assays and SRB assay"
[8]	Morais-Braga *et al*	2022	*Piper xylosteoides*	GC-MS, FID, Broth microdilution, half-maximal inhibitory concentrations
[9]	Polumackanycz *et al*.	2019	White mulberry	FRAP and DPPH assays, Ellman assay
[10]	Popova et al.	2017	*Propolis*	GC-MS chemical analysis, assays against human cancer cell lines, antifungal and antibacterial assays
[11]	Al-Hashemi *et al*	2016	*Azadirachta indica*	FCR and DPPH
[12]	Akarchariya *et al*	2017	*Curcuma* sp.	agar-well diffusion assay, and broth-micro dilution procedures
[13]	Ayaz *et al.*	2015	*Polygonum hydropiper*	Ellman's test, DPPH, ABTS and free radical scavenging assays

[Insert **Table 1** here]

2.1 Synthesized Herbal Products and Their Biological Evaluation

Curcumin[14] has anticancer and anti-chemotherapeutic properties; however, its therapeutic usefulness was constrained by its limited bioavailability. Curcumin was encapsulated in alginate aldehyde-gelatin nano-gels utilizing the inverse mini-emulsion method to address this. By using the inverse miniemulsion process, alginate aldehyde-gelatin nanogels are created. "Dynamic light scattering (DLS), NMR spectroscopy, and scanning electron microscopy (SEM)", are used to assess the properties of the "curcumin-loaded nanogels". By using the Hemolysis assay and MTT, the nanogels' hemocompatibility and cytocompatibility were assessed.

Effectively[15] encapsulated OEO in PLCL/SF nanofibers membrane (NF) and reached an encapsulation effectiveness (%) of up to 59.14 0.58. The membranes that were created were tested physicochemically as well as biologically. SEM analysis demonstrated that OEO may be successfully enclosed while retaining a smooth nanofiber profile. The cytotoxicity assay validated the NF membrane's biocompatibility. Furthermore, histological analysis was used to assess the NF membranes' ability to repair wounds in vivo. The neo epithelialization, granulation tissue development, angiogenesis, and collagen deposition were all visible using H&E and Masson's trichrome staining.

Electrospun[16] "oregano essential oil (OEO) into Poly (l-lactic acid-co-e-caprolactone)/Silk Fibroin (PLCL/SF) polymers" and estimated for numerous physicochemical and biological properties. SEM revealed the homogeneous and bead-free morphology. Diminished total reflection "Fourier transform infrared spectroscopy (ATR-FTIR)", demonstrated the effective loading of OEO. Similarly, the biological importance of the produced substance was assessed. The issue performance of OEO from electrospun membranes was explored using "liquid chromatography-mass spectrometry (LC-MS)".

Aimed[17] to green synthesize "ZnO-nanoparticles (NPs)" from several *Silybum marianum* L. Gaernt tissues which was trailed by detailed description and valuation. Thusly created "ZnO-NPs" were submitted to characterization utilizing common methods like XRD, FTIR, and SEM. Using thermo-gravimetric analysis, the thermal firmness of synthesized NPs was also

assessed. All of the synthesized "ZnO NPs" were verified for their capacity to kill human HepG2 hepatocellular cancer cells.

Examines[18] the use of the ubiquitous medicinal herb dandelion, "*Taraxacum officinale"*, in the production of "silver nanoparticles". AgNPs were examined for antibacterial efficacy against "*Xanthomonas axonopodis* and *Pseudomonas syringae"*, two significant phytopathogens. "UV-visible spectroscopy and X-ray diffraction (XRD)", were used to determine the structure of TOL-AgNPs. Phytochemicals used in the manufacture of NPs were sensed using "Fourier transform infrared spectroscopy (FT-IR)".

Investigated[19] the antibacterial efficacy of "*Berberis vulgaris* leaf and root aqueous extract- NPs". Silver nanoparticle production was carried out following "collection, identification, and extraction of *Berberis vulgaris"*. By using the Minimum Inhibitory Concentration test and the Disc diffusion test, the "antibacterial effects" of these nanoparticles on "*Escherichia coli* and *Staphylococcus aureus",* bacteria were investigated. Review of biological evaluation of synthesized herbal preparations are shown in the Table 2.

Table 2: Review of biological evaluation of synthesized herbal preparations

Citation No	Author	Year	Method Used	Sample Type
[14]	Sarika *et al.*	2016	"Inverse mini-emulsion method, Dynamic light scattering (DLS), NMR spectroscopy, and scanning electron microscopy (SEM)"	Alginate aldehyde-gelatin nano-gels loaded with curcumin
[15]	Huang *et al.*	2020	Encapsulation in PLCL/SF nanofibers membrane (NF), SEM analysis, Cytotoxicity assay, Histological analysis	Oregano essential oil (OEO)
[16]	Khan *et al.*	2019	Electrospinning, SEM, diminished total reflection "Fourier transform infrared spectroscopy (ATR-FTIR), Thermogravimetric analysis (TGA), Liquid chromatography-mass spectrometry (LC-MS)"	Poly (l-lactic acid-co-e-caprolactone)/Silk Fibroin (PLCL/SF) polymers loaded with oregano essential oil (OEO)
[17]	Abbasi *et al.*	2019	Green synthesis, XRD, FTIR, SEM, Thermo-gravimetric analysis, Antibacterial assay	Zinc oxide nanoparticles (NPs) from *Silybum marianum* (L.) Gaernt
[18]	Saratale *et al.*	2018	"UV-visible spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR)"	Silver nanoparticles (TOL-AgNPs) from *Taraxacum officinale*
[19]	Behravan *et al.*	2019	Minimum Inhibitory Concentration test, Disc diffusion test	Silver nanoparticles from "*Berberis vulgaris"*

[Insert **Table 2** here]

3. STANDARDIZATION OF HERBAL PREPARATIONS

Standardization of herbal preparations refers to the process of ensuring the consistency and quality of a given herbal product by specifying criteria for its chemical make-up, purity, and potency. This is vital since the amount of active ingredients in herbal medicines can vary significantly depending on the species of plant used, the percentage of the plant used, the region in which it was grown, and the methods employed during harvest and processing. The "transitivity and traceability system" from raw materials to finished products, together with the quality and process control models, were to be developed in order to enhance the quality of herbal products throughout the supply and production chains. The transitivity and traceability system were created based on quality markers, specifically how to control the manufacturing process in accordance with good engineering practices and how to implement risk management for quality and process control in the production of herbal medicines [20].

3.1 Parameters for Standardization of Herbal Preparations

Establishing a set of standards for the product's consistency and quality is necessary for standardizing herbal products. These factors may include the type and purity, the quantity of a specific active component, the formulation and dosage form, and the product's safety and effectiveness. Analytical techniques like chromatography, spectroscopy, or bioassays may be used

in standardization methods, depending on the particular parameters being assessed. For herbal remedies to be safe and effective, as well as to allow for consistent dosing and therapeutic effects, it is crucial to establish these parameters. Manufacturers can create high-quality products that satisfy the requirements of consumers and professionals and can be included into established healthcare systems by following to these standards.

Find[21] Chinese medicine herbs that are frequently used to treat viral respiratory infections and also contain compounds that may directly inhibit the 2019 novel coronavirus, an ongoing novel coronavirus that causes pneumonia, by conducting an appropriate test. Tests on absorption, distribution, metabolism, and excretion were performed on substances that satisfied both criteria to make sure oral delivery would work. The chemical's potential for direct 2019-nCoV protein interaction was then determined via a docking analysis. The final step was to assess the total in vivo effects of each plant using network pharmacology analysis.

Establish[22] pharmaceutical standardization of modified dosage forms of *Avalgujadi Lepguti* in the form of for convenience of administration. The was standardized in three batches, and quality assurance criteria were also investigated. The physicochemical examination covers, “loss on drying at 105 C, total ash, acid insoluble ash, alcohol soluble extractives, water soluble extractives, pH, hardness, and disintegration time”.

Because[23] of their similar morphology, the seeds of *N. sativa* are frequently adulterated with seeds of *Allium cepa* L. on the herbal market, either purposefully or unintentionally. The microscopic characterization of herbal medications was done in the current study using morphological, palynological, and anatomical aspects. Fluorescence analysis and physiochemical characteristics were used to characterize the pharmacognosis. “Moisture content, total ash, acid-insoluble ash, water-soluble ash, and water-insoluble ash”, were all measured. The aforementioned standards, which are being made public for the first time for the studied plant species, are crucial in establishing microscopic and pharmacogenetic benchmarks for the identification and certification of real herbal medicines in the future.

Focused[24] on the creation and standardization of a polyherbal capsule formulation that contains *Zingiber officinale, Piper longum, Piper nigrum, Emblica officinalis, Teriminalia chebula, Teriminalia bellerica*, and *Ocimum sanctum*. The physical properties of the poly-herbal crude drug material were analyzed for “bulk density, tapped density, compressibility index, and angle of repose”, as well as other standardization factors like organoleptic evaluation and powder microscopic analysis. The formulated poly-herbal capsule met the criteria for the attributes assessed and can be used as a benchmark for the element of quality control. Review of parameters for standardization of herbal preparations are shown in the Table 3.

Table 3: Review of parameters for standardization of herbal preparations

Citation No	Author	Year	Samples used	Methods used
[21]	Zhang *et al.*	2020	Chinese medicine herbs used for viral respiratory infections	ADME analysis, Docking analysis
[22]	Rathi *et al.*	2016	*Avalgujadi Lepguti*	Physicochemical examination
[23]	Rashid *et al.*	2018	*Nigella sativa* seeds	Microscopic characterization, Fluorescence analysis
[24]	Mahto *et al.*	2022	Polyherbal capsule formulation	Physical properties analysis, Organoleptic evaluation and Powder microscopic analysis

[Insert **Table 3**here]

3.1.1 Standardization of Certain Parameters in Herbal Products by Gas Chromatography and Spectrometry Techniques

Techniques like gas chromatography (GC) and spectrometry are frequently used to standardize specific parameters in herbal products. These methods make it possible to identify and quantify each individual chemical in a mixture, including both active ingredients and impurities. The quality and consistency of the herbal product can be preserved by creating reference standards and utilizing GC and spectrometry methods to quantify the concentration of these substances in each batch.

Utilized[25] “gas chromatography-mass spectrometry and chiral gas chromatography”, to examine the chemical composition of the rosemary oils. “(+) --pinene (13.5%–37.7%), 1,8-cineole (16.1%–29.3%), (+)-verbenone (0.8%–16.9%), ()-borneol (2.1%–6.9%), ()–camphor (0.7%–7.0%), and racemic limonene (1.6%–4.4%)”, made up the majority of the oils. A hierarchical cluster analysis revealed that rosemary oil has at least five distinct chemotypes.

Examined[26] the phytochemical profiles and biological activities of the three-cardamom species' essential oils—*Elettaria cardamom, Aframomum corpora*, and *Amomum subulatum*—to shed light on any potential differences in the biological activities of the three-cardamom species' essential oils. The oxygenated monoterpenes made up the majority, which were examined using the "GC and GC/MS techniques".

The *Hydrocotyle javanica* Thunb[27]standardization leaves were validated using step-by-step "physicochemical tests, element analysis, determination of ash values, fluorescence analysis, evaluation of moisture content, extractive values in various solvent systems, and extraction techniques". "Atomic absorption spectrophotometry, an inductively coupled plasma-mass spectrometer, and a CHNS/O analyzer", were used to assess the heavy metal, mineral, and element contents. The facility under test had a heavy metal profile that was within the allowable limits set by the regulatory agencies. Review of gas chromatography and spectrometry techniques in standardization are shown in the Table 4.

Table 4: Review of gas chromatography and spectrometry techniques in standardization

Citation No	Author	Year	Samples Used	Methods Used
[25]	Satyal *et al.*	2017	Rosemary oils	"Chiral gas chromatography and gas chromatography-mass spectrometry Hierarchical cluster analysis"
[26]	Noumi *et al.*	2018	Essential oils of *Elettaria cardamom, Aframomum corpora,* and *Amomum subulatum*	Gas chromatography and gas chromatography-mass spectrometry
[27]	Mandal *et al.*	2017	*Hydrocotyle javanica* Thunb standardization leaves	Physicochemical tests, fluorescence analysis and AAS

[Insert **Table 4** here]

3.1.2 Standardization of Certain Parameters in Herbal Products by Liquid Chromatography Techniques

Standardizing aspects of herbal products, including the concentration of particular active components, can be done using liquid chromatography (LC) techniques. LC is a potent analytical technique that allows for accurate assessments of active substances by separating and quantifying each component in a mixture. The quality and consistency of the herbal product can be preserved by creating a reference standard and utilizing LC to quantify the concentration of active components in each batch. This process of standardization makes sure that each batch of the medicine contains a same quantity of active ingredients, enabling consistent dosing and effectiveness.

Utilizing *Adhatoda vasica*,[28]a thorough plan was established in polyherbal compositions. "Identification of marker compounds in polyherbal products, isolation, purification, and characterization of the marker compounds, and MRM-based quantitative analysis of the isolated marker compounds using LC-ESI-MS/MS", are all examples of untargeted metabolite profiling using LC-ESI-MS/MS that is used to analyze plant samples for unintended metabolites. *A. vasica* was confirmed to be present in intricate polyherbal compositions through the identification of distinctive peaks by chemical fingerprinting of the plant.

Organized[29] the PLD Kashaya formula by identifying physical and chemical properties, screening for phytochemicals, and creating a TLC fingerprint profile. For the first time, the PLD Kashaya formula was standardized, and the output of the existing study's findings were utilized as a reference standard for the medication's quality control. The dichloromethane extract of the Panchamuli PLD Kashaya formula's TLC fingerprint profile was created.

"A targeted liquid chromatography (LC)[30] in tandem with tandem mass spectrometry approach", was developed to examine the presence of "coumarins and furocoumarins" in essential oils and plant extracts, using 39 reference standards. Chromatographic separation was achieved using a reversed phase column with water/acetonitrile as the mobile phase, while detection was performed using a hybrid spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source that worked in positive ion mode. This analytical method was applied to investigate the coumarin composition of fruit essential oils and methanolic extracts obtained from different parts of *Zanthoxylum zanthoxyloides*.

Examined[31] a TCM remedy named *Yindan xinnaotong* soft pill (YDXNT), which is made up of 8 plants to identify potential neuroprotective components. To confirm the purity of YDXNT, the researchers used a variety of chromatographic techniques and bioactivity experiments to find 124 compounds. They then chose potential ECCs based on their absolute concentrations and neuroprotective properties. Chemical fingerprints were examined using "gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) techniques". Then, using dual phenotypic quantification based on images, "the neuroinflammation activities of YDXNT chemical library members", were determined. In order to identify the possibly active chemicals with interaction effects, "fingerprint-efficacy correlation and random forest analysis", were employed.

Set[32] up criteria for *Ashwagandha lehyam* standardization. evaluation of different standardization criteria, including "organoleptic characteristics, physico-chemical evaluation, finger print profiling using HPTLC and estimation of bioactive markers, including withaferin-A", by HPLC. Bioactive indicators are traits of the components or botanicals that make it simple to recognize their presence in a formulation. "The confirmation through the HPTLC fingerprint profile and quantification of biomarker by HPLC", are the best ways to identify and appraise the eminence of the preparation.

In addition[33] to artemisinin, preparations of *Artemisia annua* may possibly contain other mixtures with antitumor prospective. The extract, 1H-, and 13C were all used to characterize it using "HPLC-DAD". The most common complexes were detected and measured using UHPLC-MS/MS and NMR spectroscopy. Cell viability and a number of apoptotic traits were assessed using flow cytometry. "The chick chorioallantoic membrane (CAM) assay and orthotopic breast cancer xenografts in nude mice", were utilized to validate in vitro results.

Formulated[34] two analytical procedures based on fingerprint and colour measurements. Spectrophotometric evaluation was used to assess the colour standards of the *Crataegi fructus* peel during the colour measurements. Then, mathematical formulas were developed to estimate the level of processing after the colour values of "*Crataegi fructus"* and its processed products were analyzed using, "Bayes linear discriminant analysis". "A Hibar C18 column was also used for high-performance liquid chromatography fingerprint analysis".

Established[35]several pharmacognostic and phytochemical standards that can be used to guarantee the medicinal plant *G. trichophylla*'s purity, safety, and effectiveness. The tools for differentiating the powdered drug material revealed to be histochemical, phytochemical, physicochemical, and fluorescence examination. The presence of significant phytoconstituents such "gallic acid, rutin, and quercetin", was detected using "high-performance liquid chromatography (HPLC)" examination.

Piper nigrum L[36]. fruit should go through HPLC fingerprinting to standardize the characteristics to determine the typical pharmacognostic of the "king of spices" and looked at "the extractive values, total ash value, water soluble ash value, acid insoluble ash value, moisture content, loss on drying, and pH values" in the *P. nigrum* L. fruits. "The Harborne approach" was applied to the initial screening and "total phenolic and flavonoid levels, heavy metal concentrations, pesticide residues, and aflatoxin levels", were all examined. Using the CAMAG-high performance thin layer chromatography method, the methanolic extract of P. nigrum L. fruits was used for fingerprinting.

Analyses[37] the effects of the Ridayarishta formulation on the human hepatic cytochrome P450 (CYP450) enzyme by an experiment to measure herb-drug interactions (HDI). Using "high-performance thin-layer chromatography (HPTLC)" analysis, the "Ridayarishta formulation", was phytochemically standardized against "arjunolic acid, arjunetin, berberine, piperine, resveratrol, and withaferin-A". Gas chromatographic (GC) analysis was used to standardize the formulation's ethanol concentration.

Described[38] the *Pathyashadangam kwath*'s distinctive characteristics to attest to its high caliber and purity. According to accepted procedures, the kwath was assessed for organoleptic, physical, phytochemical, and chromatographic parameters. Andrographolide was found to be an acceptable marker for the kwath's standardization by HPLC analysis. *Pathyashadangam kwath* quality control analysis using this study may become common practice.

Compared[39] the antioxidant capacity and acetylcholinesterase (AChE) inhibitory activity of commercial white mulberry samples' phenolic profiles. It would be interesting to know whether the phenolic profiles of herbal products coming from various commercial sources vary. The documentation of ten main phenolic compounds was accomplished for this purpose using a quick and easy HPLC approach.

To[40] analyze the chemical components of GDD, "a dependable and sensitive method based on ultra-performance liquid chromatography combined with quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MSE) and the UNIFI

informatics platform", was used. By comparing their retention times, accurate masses within 5 ppm error, and MSE fragmentation patterns, 96 compounds from the GDD were discovered or provisionally characterized, including "anthraquinones, alkaloids, protostane triterpenoids, flavonoids, and triterpenoid saponins". The fragmentation patterns and distinctive ions of representative compounds with various chemical structure types were examined.

The[41] chemical components in YXST were thoroughly analyzed using "high-performance liquid chromatography coupled with electrospray ionisation quadrupole time-of-flight mass spectrometry (HPLC-ESI-Q-TOF-MS)". "The 1,1-diphenyl-2-picrylhydrazyl (DPPH) spectrophotometric test", was used to demonstrate the antioxidant activity of YXST, and the DPPH-HPLC experiment was used to quickly screen the antioxidant content of YXST.

Haemorrhages,[42] cough, asthma, and hair loss can all be treated with "Platycladi Cacumen (PC), a traditional Chinese medicine". In order to better understand the chemical makeup of PC, "ultra-high performance liquid chromatography", was first used in conjunction with "electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS) and a diagnostic ion filtering strategy". "The chemical variation of 39 batches of PC from diverse geographic sources and 10 batches of PC-processed goods", was further examined by evaluating the concentrations of nine essential flavonoids. Review of liquid chromatography techniques in standardization are shown in the Table 5.

Table 5: Review of liquid chromatography techniques in standardization

Citation No.	Author	Year	Sample used	Methods used
[28]	Rahman *et al.*	2019	Plant samples in polyherbal products	LC-ESI-MS/MS for untargeted metabolite profiling
[29]	Wickramaarachchi *et al.*	2016	PLD Kashaya formula	Phytochemical screening, TLC fingerprint profile
[30]	Tine *et al.*	2017	Essential oils and plant extracts	LC-MS/MS for targeted analysis of coumarins
[31]	Pang *et al.*	2019	TCM remedy (YDXNT)	GC-MS and LC-MS for chemical fingerprint analysis
[32]	Abraham *et al.*	2020	*Pathyashadangam kwath*	HPLC for andrographolide marker standardization
[33]	Meena *et al.*	2021	Ashwagandha lehyam	HPTLC fingerprint profiling and HPLC for markers
[34]	Lang *et al.*	2019	*Artemisia annua* extracts	HPLC-DAD, UHPLC-MS/MS, NMR spectroscopy
[35]	Fei *et al.*	2018	*Crataegi fructus* and processed forms	Spectrophotometric color measurement, HPLC
[36]	Alam and Najum us Saqib	2015	*G. trichophyllum*	Pharmacognostic and phytochemical standards
[37]	Ahmad *et al.*	2015	*Piper nigrum* L. fruits	High-performance thin-layer chromatography (HPTLC) and Harborne approach
[38]	Pandit *et al.*	2017	Ridayarishta formulation	High-performance thin-layer chromatography (HPTLC)
[39]	Polumackanycz *et al.*	2019	Commercial white mulberry samples	HPLC approach
[40]	Xu *et al.*	2020	GDD	Ultra-performance liquid chromatography (UPLC-Q-TOF-MSE)
[41]	Zhu *et al.*	2017	YXST	"HPLC coupled with electrospray ionisation quadrupole time-of-flight mass spectrometry (HPLC-ESI-Q-TOF-MS)"

[42]	Zhuang *et al.*	2018	Platycladi Cacumen (PC)	"Ultra-high performance liquid chromatography in conjunction with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS)"

[Insert **Table 5** here]

3.1.3. Standardization Of Herbal Products Based on Molecular Parameters

Standardization of herbal products based on molecular parameters involves the detection of specific molecular markers in the herbal product. These markers are chosen based on their association with the "efficacy and safety" of the herbal product. By establishing the concentration of these molecular markers, the quality and consistency of the herbal product can be maintained. This method of standardization ensures that each batch of the product contains a consistent number of active compounds, allowing for reliable dosing and efficacy. Additionally, it allows for greater control over the eminence and security of the product, reducing the risk of adulteration and contamination.

Designed[43] DNA barcoding for Lamiaceae medicinal plants to ensure their accurate detection and to address the issue of contamination to safeguard customers from health concerns brought on by product contamination and substitution. Many Lamiaceae species were utilized as herbs, spices, and sources of essential oils in cooking as well as traditional medicine. It is now conceivable to detect the herbs and detect additives in herbal medicinal goods thanks to a scientific technology called DNA barcoding.

Strict[44] guidelines for material handling and the authenticity and similarity of the therapeutic ingredients are important components to maintain the quality of herbal remedies. The process of authentication was revolutionized by using genome-based techniques to authenticate these plants. The most effective method to distinguish adulterants from real medicinal plant species is to create DNA molecular markers by sequencing a standard region of the DNA. The use of molecular biological techniques, such as the PCR technique, the most reliable approaches for verifying the authenticity of herbal preparation, according to *Glycyrrhiza glabra* L.

To[45] guarantee the excellence of herbal medicines, it's important that the medicinal ingredients be true to form, identical in composition, and handled with stringent protocols. The authentication procedure was transformed by genome-based technologies to identify these plants. The best method to distinguish between adulterants and legitimate specimens of the required species of medicinal plants is to create DNA molecular markers by sequencing a standard zone of the DNA. As stated in *Glycyrrhiza glabra* L., one of the extremely reliable methods for authenticating natural herbal materials is the use of molecular biological techniques like the PCR method.

Employed[46] DNA barcode analysis to identify the common adulterants and each herbal remedy at the species level. In order to confirm the authenticity of these herbal remedies, researchers gathered six common adulterant plant species together with five genuine species (*Pinellia ternata* and *Arisaema amurense*,). The phylogenetic tree produced by the amplification of the "matarase K (matK) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) genes", revealed nine different groups that differed by species.

Described[47] an instance in which DNA barcoding was used to identify the species of market samples of *Sida cordifolia*. 13 species of *Sida* were gathered as a preliminary to species identification by DNA barcoding, and "a reference DNA barcode library", was created utilizing "the rbcL, matK, psbA-trnH, and ITS2 markers".

Based[48] on the ITS2 sequencing, "41 *Herba anoectochili* and its adulterant samples", were determined. "A specific primer was created based on the ITS2 sequence and a real-time PCR detection system", was established for the swift, sensitive, and specific identification of *Herba Anoectoch*, as shown by "the sequence characteristics, Basic Local Alignment Search Tool (BLAST) application, genetic distance, construction of a phylogenetic tree, secondary structure prediction, and other methods".

"Three fluorescently-labeled peptide nucleic acid (PNA) probes", were created against "ITS2 sequences that contained single nucleotide polymorphisms (SNPs)" and utilized in "a real-time PCR melting curve experiment"[49] to progress a technique for recognizing the four *Paeonia* species used in these medications. This investigation allowed for the precise identification of each of the four *Paeonia* species. Review of molecular parameters-based standardization are shown in the Table 6.

Table 6: Review of molecular parameters-based standardization

Citation No	Author	Year	Samples used	Methods used
[43]	Zahra *et al.*	2016	Lamiaceae medicinal plants	DNA barcoding, sequencing a standard region of the DNA, molecular biological techniques like PCR
[44]	Butt *et al.*	2018	*Glycyrrhiza glabra* L	DNA molecular markers
[45]	Han *et al.*	2016	Medicinal plants	DNA barcoding, sequencing a standard zone of the DNA, molecular biological techniques like PCR
[46]	Moon *et al.*	2016	Adulterant plant species and genuine species	DNA barcode analysis, sequencing the matK and rbcL genes, phylogenetic tree
[47]	Vassou *et al.*	2015	Market samples of *Sida cordifolia* and 13 species of Sida	DNA barcoding, reference DNA barcode library creation using rbcL, matK, psbA-trnH, and ITS2 markers
[48]	Shuai-Jun *et al.*	2019	*Herba anoectochili* and its adulterant samples	ITS2 sequencing, specific primer creation, real-time PCR detection system, sequence characteristics, BLAST application, genetic distance, phylogenetic tree
[49]	Kim *et al.*	2017	Four *Paeonia* species	Fluorescently-labeled PNA probes, real-time PCR melting curve experiment, sequencing of ITS2 sequences containing SNPs

[Insert **Table 6** here]

4. CHALLENGES TO THE STANDARDIZATION OF HERBAL PREPARATIONS

Herbal medications are often less harmful than allopathic medicines, according to adverse reaction data compiled in the literature. However, for herbal medicines to be a viable therapeutic alternative in the modern period, they must meet contemporary safety requirements. Some natural goods that include herbal ingredients can be harmful to consumers. Assuming that all products are safer than synthetic drugs is false. Every substance that modifies the body's defense mechanisms or physiology, however, may lead to varying degrees of adverse effects. Despite the dangers connected with a variety of traditional medicinal herbs with documented risks, insufficient quality controls, erroneous prescriptions and administration, and a lack of safety procedures lead to numerous safety issues. Because it has been discovered that numerous botanicals contain hazardous chemicals in quantities dangerous to human health, this generalization is oversimplified. Plants with acute toxicity are typically already known to be dangerous due to their traditional uses, while those with subacute and chronic toxicity are difficult or even impossible to identify by conventional use or clinical research investigations.

The safety of traditional herbal medicines has been a growing concern due to the potential contamination with pyrrolizidine alkaloids (PAs), which can cause liver damage. [49]conducted a study to determine the content of PAs in 70 commonly used plant-derived medicinal products sold in "Ghana and other West African countries". Their aim was to assess the possible health risks associated with these products. Meanwhile,[50][51]investigated the consequence of different sample research methods on the mechanisms and liver toxicity of extracts derived from *P. multiflorum*. The study found that the method used to prepare the extracts had a significant impact on the components present in the extracts, particularly those related to liver toxicity. These findings highlight the importance of using appropriate sample preparation methods to obtain accurate and reliable results in toxicity studies.

Additionally, [51]evaluated the critical lethal ability of an "aqueous ethanolic extract of *S. munja* roots", using the OECD TG No. 425 guideline. Their study intended to control the safe levels of use of the extract in traditional medicine and prevent adverse health effects in consumers. These studies demonstrate the need for proper evaluation of the safety of herbal medicines before their use to prevent potential harm to human health.

5. CONCLUSION

For centuries, herbal preparations have been utilized to prevent and treat a diverse range of diseases and conditions. Nonetheless, the safety and efficacy of these preparations can be influenced by several factors, including the selection of plant material, preparation techniques, and formulation standardization. The selection of appropriate plant material is a serious factor in the "quality and safety" of herbal preparations. It is vital to ensure that the plant material used is of high value, free from contaminants, and properly identified. The use of incorrect plant material or adulterated material can result in adverse effects and reduced efficacy. The preparation method of herbal preparations is also crucial in determining their biological activity. Different methods of extraction can yield different concentrations of active compounds, which can influence the efficacy and safety of the preparation. For example, a preparation that is not properly extracted or standardized can result in varying potency or concentration of active compounds, which may lead to ineffective treatment or adverse effects.

Standardization of herbal preparations is important to confirm consistent "quality and efficacy". Standardization includes the determination of the concentration of active compounds in the preparation, as well as the establishment of specifications for purity, identity, and potency. The use of standardized herbal preparations can help to ensure consistent efficacy and safety, as well as provide a basis for comparison between different preparations. Scientific validation of herbal preparations is essential to provide evidence for their efficacy and safety. The use of "in vitro and in vivo models", can help to clarify the mechanisms of herbal preparations, as well as determine their potential toxicity. Clinical studies can provide further evidence of efficacy and safety in humans. In conclusion, the development of standardized and scientifically validated methods for the preparation and evaluation of herbal preparations is critical to ensure their safety and efficacy. This can help to increase the acceptance and integration of herbal medicine into mainstream healthcare, providing patients with more effective and personalized treatment options. It is important to continue to research and develop new methods for the preparation and evaluation of herbal preparations to improve their quality and safety.

6. DECLARATIONS

ETHICS APPROVAL

No ethics approval is required.

7. HUMAN AND ANIMAL ETHICS

Not Applicable.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

All the data is collected from the simulation reports of the software and tools used by the authors. Authors are working on implementing the same using real world data with appropriate permissions.

AUTHOR'S CONTRIBUTIONS

Author 1: Mr. Mohit Kumar

He performed the conceptualization, Methodology, Data collection and writing the study

Author 2: Dr. Charu Bharati

He analysis the dataset and conceptualization in the study.

Author 3: Dr. Reetu Yadav

He Performed the Analysis of overall concept, writing and editing.

Author 4: Dr. Ashutosh Yadav

He analysis the paper and supervisor of this paper.

Author 5: Mr. Nitin Kumar

He participated in the methodology, Conceptualization,.

Author 6: Ms. Rabiya Praveen

He participated in the Data collection and writing the study

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CONFLICTS OF INTEREST

The authors declare that we have no conflict of interest.

COMPETING INTERESTS

The authors declare that we have no competing interest.

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