



International Journal of Research Publication and Reviews

Journal Homepage: www.ijrpr.com ISSN 2582-7421

Metabolomics-Based Quality Markers for Standardization of Herbal Formulation

Aniket Uttam Mohite

Ashok Rao Mane Institute of Pharmacy, Ambap

ABSTRACT

Herbal plants are found abundant in all parts of the world with various uses for its medicinal, pharmaceutical, and nutritional value as well as the ordinary spice in our culinary. Nature is a principal source of herbal medicines. The increasing use of herbal medicine around the globe requires new scientific approaches for their standardization. Evaluation of Physical and chemical parameters are the most common parameters for standardization. Now days, a number of method exists, but not satisfying everyone all over the globe. With the use of newer technique such as omics, (proteomics, metabolomics, transcriptomics etc.), we can easily have depth knowledge of pharmacodynamics, pharmacokinetics and toxicological characterization of active constituent of an herbal plant. These techniques have made one standard approach for the standardization and modernization of herbal medicine. The modernization of herbal medicine is the need of hour to cater the global demand by means of patient compatibility. The omic technique can also play an important role for the conservation of knowledge of ancient plant species. Moreover omic technique is an important tool for fingerprinting and quality control of herbal medicine. In this review, an emphasis has been made on different techniques of omics and their use in standardization and modernization of herbal plant.

1.INTRODUCTION

Throughout history, all societies have utilized herbal medicines as a delicate, age-old kind of healthcare. The first humans discovered useful plants through trial and error. A crucial prerequisite for quality control and dose calculation of plant-related drugs is the identification of the pure active moiety. When herbal medications are standardized, their identification, quality, and purity are verified. The standardization parameters and their standard values for a few herbal medications are covered in this overview. Approximately 85% of the global population receives their medical care from conventional medications. Maintaining the plant's and its products' efficacy, safety, and quality is crucial to preventing major health issues. [1] Ayurveda continues to be more prevalent in Indian healthcare than contemporary medicine, especially when it comes to treating a wide range of chronic illnesses. [2] Plant-based products have become more popular in recent years as medicines, nutraceuticals, and cosmetics. They can be purchased over-the-counter in pharmacies and health food stores as self-medication or as prescription drugs in non-allopathic systems [3, 4]. [5] Because they are poorly absorbed when taken orally, herbal medications, which are widely used in healthcare in both developed and developing nations, are complex chemical mixes made from plants and have limited efficacy [5]. Approximately 80% of people worldwide still utilize herbs and other traditional medicines for their basic medical needs, according to a World Health Organization (WHO) estimate [6]. Herbal compositions are now widely accepted as medicinal agents for liver illnesses, diabetes, arthritis, cough cures, memory enhancers, and adaptogens [7]. Raw plant material, processed plant material, and therapeutic herbal products are the three categories of herbal medicines according to the WHO. A. Herbal medicines are finished goods with labels that include active substances, such as plant material or aerial or subterranean plant parts, either in their raw form or as plant preparations. In keeping with the global trend of people going back to natural therapies, the usage of herbal medications has significantly increased [8]. Herbal medicine products, which come in the form of tablets, capsules, powders, teas, extracts, and fresh or dried plants, are dietary supplements that people consume to enhance their health [9].

Due to the inherent batch-to-batch variability in biological material, the finished products should ideally be standardized, which implies that they should be adjusted to a specified content of elements with established therapeutic action as far as this is practical. Pattern-oriented methods based on fingerprints are emerging tools that are thought to be very helpful for the quality control of herbal products in addition to quality control methods based on chemical markers. This is because these methods are thought to be better able to depict the complex nature of phytochemical preparations than the more conventional marker-based methods. A. Proper starting material quality and manufacturing quality at every stage can be ensured in a variety of ways. For every action, a different approach must be selected depending on the equipment available and the task at hand. Many pharmacopoeia monographs offer methods for quantitative measurements based on HPLC and sometimes GC, as well as identification techniques based on macroscopic, microscopic, and TLC. Despite being dependable, quick, and affordable, these methods won't be sufficient for every application. [10]

The term "herbal drugs" refers to plants or portions of plants that have been transformed into phytopharmaceuticals through simple collecting, harvesting, drying, and storage procedures [11]. Herbal remedies have long been used to treat and prevent illnesses. Many civilizations have a secular tradition of using medicinal herbs for therapeutic purposes [12,13]. Their application dates back to the earliest recorded accounts in various Ayurvedic books. Human

civilization has always included them. According to the WHO, almost 80% of people on the planet still get their primary medical treatment from medicinal herbs. The usage of herbal medicine is growing daily not only in India but also in western countries [14].

2. Quality Control Challenges in Herbal Medicines

2.1 Variability in herbal raw materials

The phytochemical composition of herbal raw materials is greatly influenced by environmental conditions, including soil composition, climate, and altitude. Changes in these circumstances may result in variations in the active chemical concentration, which will ultimately affect the herbal product's effectiveness. For example, because to variations in environmental stresses and nutrient availability, the same species of plant grown in different locales may produce extracts with varied medicinal qualities. Furthermore, variations between plant species or subspecies can lead to inconsistent quality, a phenomenon known as intra-species variability. Manufacturers seeking to create standardized herbal products face difficulties due to this diversity, which also makes quality control efforts more difficult. [15]

2.2 Complexity of herbal formulations

Multiple active and inert components are frequently included in herbal preparations, which complicates quality control. Although the existence of different constituents can result in unanticipated interactions between distinct components, it can also contribute to synergistic effects that improve therapeutic efficacy. These interactions may change the formulation's overall efficacy and bioavailability, making it challenging to determine reliable results across batches. Furthermore, the complexity of these mixes makes analytical evaluations more difficult because conventional techniques might not fully capture the complex interactions present in the formulations.

2.3 Lack of standardized manufacturing procedures

One major obstacle to guaranteeing consistent quality in herbal medications is the absence of established production processes. Conventional processing methods frequently differ significantly between manufacturers, resulting in differences in the potency and content of the final product. Even while contemporary herbal processing procedures strive to increase consistency, differences in raw material quality and extraction techniques make it difficult to maintain batch-to-batch uniformity. The chemical profile of the finished product can be affected by variables including extraction solvents, temperatures, and times. As a result, obtaining trustworthy quality control in the herbal medicine sector continues to be extremely difficult in the absence of established procedures. [16]

3. Herbal drug standardization

A procedure known as standardization guarantees a predetermined quantity, purity, and therapeutic impact of substances in every dosage [17]. If the medicine tested has not been validated and characterized to guarantee reproducibility in the product's manufacturing, the herbal product cannot be regarded as scientifically valid. Furthermore, a number of potentially fatal side effects, including as direct toxic effects, allergic reactions, impacts from pollutants, and interactions with herbal medications, have been documented recently [18]. The phytochemical components of a herbal preparation determine its therapeutic efficacy. One of the biggest challenges facing scientists is the development of legitimate analytical techniques that can accurately profile the phytochemical content, including quantitative studies of marker/bioactive molecules and other important elements. Considering the aforementioned, standardization is a crucial stage in establishing a uniform chemical profile, biological activity, or just a quality assurance program for the manufacturing and production of a herbal medication [19]. Pharmaceutical enterprises, public health, and the reproducible quality of herbal medicine all depend on the authenticity of herbal medications and the identification of adulterants from real medicinal herbs [20].

3.1 Conventional methods for standardization of herbal formulation

The passport information of raw plant medicines, botanical authentication, microscopic and molecular analysis, identification of chemical composition using different chromatographic techniques, and biological activity of the entire plant are all included in the standardization of herbal raw medications. Numerous workers have reported on the macroscopic and microscopic assessment and chemical profile of the herbal materials for quality control and standardization [21, 22]. While microscopy entails a comparative microscopic examination of powdered herbal medication, macroscopic identification of medicinal plant materials is dependent on sensory evaluation factors such as shape, size, color, texture, odor, and taste. Furthermore, because light and scanning electron microscopes (SEM) are used in the standardization of herbal drugs, developments in microscope technology have improved the accuracy and capacities of microscopy as a means of identifying herbal crude materials. Additionally, a number of sophisticated techniques are presently used in the standardization of herbal medications, including chromatographic, spectrophotometric, and combinations of these techniques, electrophoresis, polarography, and the application of molecular biomarkers in fingerprints [23,22,24-28].

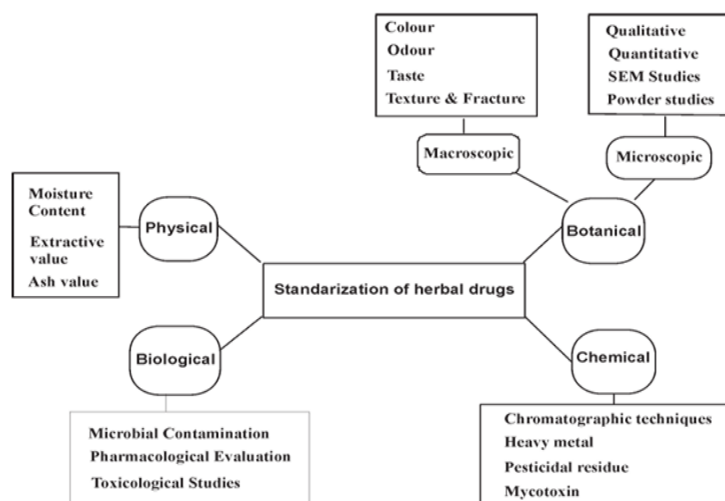


Figure 1: A schematic representation of herbal drug standardization

3.2 Standardization of polyherbal formulations

Since polyherbal formulations combine multiple herbs to achieve the desired therapeutic effect, standardization is crucial for preserving and evaluating the quality and safety of these formulations [29]. Organoleptic, physical, and physico-chemical features have been used to standardize the polyherbal formulation of hyperlipidemia [30]. A US patent has been obtained for the creation and standardization of a polyherbal formulation (Artrex®) that contains four botanicals and is intended to treat arthritis. This process was carried out utilizing contemporary scientific instruments and known indicators [31].

Momordica charantia (seeds), Syzigium cumini (seeds), Trigonella foenum (seeds), Azadirachta indica (leaves), Emblica officinalis (fruits), Curcuma longa (rhizomes), Gymnema sylvestre (leaves), and Pterocarpus marsupium (heart-wood) are the eight herbal antidiabetic drugs that make up Madhumehari Churna (Baidynath). There have been reports of Gokshuradi Churna, Megni, Jawarish-e-Darchini [35–37], Pancasama Churna, which is known to be beneficial in gastrointestinal disorders [33], and Dashamularishta, a traditional formulation utilized in the normalization of physiological processes following childbirth [34]. However, many polyherbal formulations still need to be standardized because they are often employed solely for ethanobotanical purposes [38]. Standardization ensures the polyherbal formulations' safety, efficacy, quality, and acceptability while reducing batch-to-batch variance [39].

3.3 Guidelines for the standardization of herbal drugs

The following WHO guidelines are adhered to: a) botanical characteristics, sensory evaluation, foreign organic matter, microscopic, histological, histochemical assessment, quantitative measurements; b) physical and chemical identity, fingerprint chromatography, ash values, extractive values, moisture content, volatile oil and alkaloids tests, quantitative estimation protocols; c) estimation of biological activity, the values of bitterness, astringency hemolytic index, a factor swelling, heavy metals, microbial contamination as viable count total, pathogens like *E. coli*, *Salmonella*, *P. aeruginosa*, *S. aureus*, and *Enterobacteriaceae*; and e) microbial contamination and radioactive contamination.

4. Techniques in extraction of herbals

4.1 Supercritical fluid extraction (SFE)

The best method for removing the bioactive compound from aromatic and medicinal plants is supercritical fluid extraction (SFE) [41]. SFE has become a very promising method for producing nutraceuticals and herbal medications with potent active ingredients [42]. The necessary phytoconstituents have been successfully separated from the herbal extracts using SFE procedures [43].

4.2 Microwave-assisted extraction (MAE)

High-value substances from natural sources, such as phytonutrients, nutraceutical and functional food ingredients, and pharmaceutical actives from biomass, are extracted using MAE technology [44]. Carotenoids from single cells, taxanes from *taxus* biomass, essential fatty acids from microalgae and oilseeds, phytosterols from medicinal plants, polyphenols from green tea, and essential oils from a variety of sources can all be extracted using MAE. This approach has the following advantages over traditional solvent extraction methods: a) better product; -purity of crude extracts; -stability of marker chemicals; and -use of fewer hazardous solvents. b) lower processing costs, higher marker compound recovery and purity, extremely quick extraction rates, and lower energy and solvent consumption [45, 46]

4.3 Solid phase extraction (SPE)

Analytes are separated from a liquid matrix and purified herbal extracts using the SPE technique. Compared to more conventional sample preparation methods, this method offers numerous benefits, including high analyte recoveries, analyte concentration, highly purified extracts, the capacity to simultaneously extract analytes of a wide polarity range, ease of automation, compatibility with instrumental analysis, and a decrease in organic solvent [47].

5. Herbal Drug Standardization

A procedure known as standardization guarantees a predetermined quantity, purity, and therapeutic impact of substances in every dosage [48]. If the medicine tested has not been validated and characterized to guarantee reproducibility in the product's manufacturing, the herbal product cannot be regarded as scientifically valid. Furthermore, a number of hazardous and fatal side effects, including as direct toxic effects, allergic reactions, impacts from pollutants, and interactions with herbal medications, have been documented recently. Sixth. The phytochemical components of a herbal preparation determine its therapeutic efficacy. One of the biggest challenges facing scientists is the development of legitimate analytical techniques that can accurately profile the phytochemical content, including quantitative studies of marker/bioactive molecules and other important ingredients. Given the aforementioned, standardization is a crucial stage in establishing a consistent chemical profile, biological activity, or just a quality assurance program for the manufacturing and production of a herbal medication [49].

5.1 Conventional methods for standardization of herbal formulation

The passport information of raw plant medicines, botanical authentication, microscopic and molecular analysis, chemical composition identification using different chromatographic techniques, and biological activity of the entire plant are all part of the standardization of herbal raw medications [50]. Numerous researchers have documented the macroscopic and microscopic assessment and chemical profile of the herbal materials for quality control and standardization [51–53]. While microscopy entails a comparative microscopic examination of powdered herbal medication, macroscopic identification of medicinal plant materials is dependent on sensory evaluation factors such as shape, size, color, texture, odor, and taste. Furthermore, because light and scanning electron microscopes (SEM) are used in the standardization of herbal drugs, developments in microscope technology have improved the accuracy and capacities of microscopy as a means of identifying herbal crude materials¹⁸. Additionally, a number of sophisticated techniques are presently used in the standardization of herbal medicines, including chromatographic, spectrophotometric, and combinations of these techniques, electrophoresis, polarography, and the use of molecular biomarkers in fingerprints [50], [53], [54–58].

5.2 Standardization of herbal formulation

Good Manufacturing Practices (GMP) must be used in order to standardize herbal formulations [51–53]. Furthermore, it is thought to be crucial to investigate a number of factors, including pharmacodynamics, pharmacokinetics, dose, stability, self-life, toxicity assessment, and chemical profiling of the herbal formulations [55]. Equally significant are other elements like pesticide residue, aflatoxine concentration, heavy metal contamination, and Good Agricultural Practices (GAP) in the standardization of herbal drugs [59].

5.3 Standardization of polyherbal formulations

Since polyherbal formulations combine multiple herbs to provide the desired therapeutic effect, standardization is crucial for maintaining and evaluating the quality and safety of the formulation [60]. Based on physical attributes, physico-chemical properties, and organoleptic qualities, the polyherbal formulation of hyperlipdemia has been standardized [61]. A US patent has been obtained for the creation and standardization of a polyherbal formulation (Artrex®) that contains four botanicals and was created for the treatment of arthritis utilizing contemporary scientific instruments and recognized markers [62]. *Momordica charantia* (seeds), *Syzigium cumini* (seeds), *Trigonella foenum* (seeds), *Azadirachta indica* (leaves), *Emblica officinalis* (fruits), *Curcuma longa* (rhizomes), *Gymnema sylvestre* (leaves), and *Pterocarpus marsupium* (heart-wood) are the eight herbal antidiabetic drugs that make up *Madhumehari Churna* (Baidynath). [63].

5.4 DNA fingerprinting technique

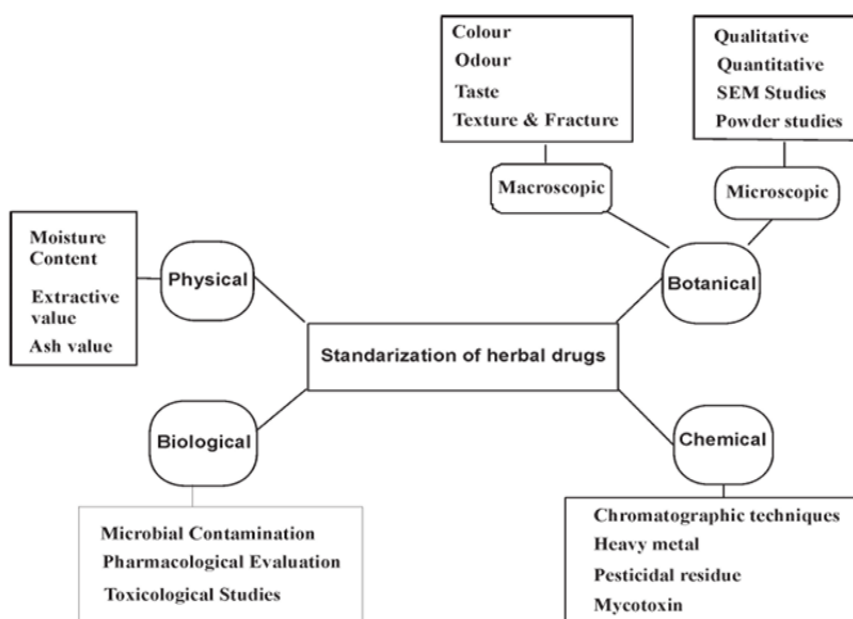
It has been demonstrated that DNA analysis is a crucial technique for standardizing herbal drugs. This method works well for identifying phytochemically indistinguishable authentic drugs from counterfeit or substituted drugs. According to reports, the DNA fingerprint genome is the same regardless of the plant part employed, but the phytochemical content varies depending on the plant part, physiology, and environment [64]. The availability of intact genomic DNA specificity in marketed herbal medications, which aids in identifying adulterants even in processed samples, is another practical use of DNA fingerprinting [65]. The relationship between DNA markers and phytochemical composition across closely related species has been the subject of numerous investigations in recent years [66]. Random amplified polymorphism and random fragment length polymorphism DNA markers have been used to report interspecies variation in various taxa, including *Glycyrrhiza*, *Echinacea*, *Curcuma*, and *Arabidopsis* [67].

5.4.1 ISSR (Inter-Simple Sequence Repeat)

ISSR, a PCR-based application, is a novel and widely used DNA fingerprinting technology that includes gene tagging, clonal variation identification, phylogenetic analysis, genomic instability detection, hybridization assessment, and genetic fingerprinting characterisation [68]. ISSR markers have been used to distinguish *Cannabis sativa* and *Arabidopsis thaliana* L. Heyne from their contaminated species [67]. Herbs can be effectively and reliably authenticated and distinguished from their adulterants using molecular analysis using Sequence-characterized amplified region (SCAR) markers. Additionally, SCAR markers can be used to distinguish between physically similar plant species [68]. Different accessions of *Taxus wallichiana*, *Azarchdichta indica*, *Juniperus communis* L., *Codonopsis pilosula*, *Allium schoenoprasum* L., and *Andrographis paniculata* collected from various geographical regions have been proven to be distinguishable using DNA-based genetic markers [69].

5.5 Guidelines for the standardization of herbal drugs

The following WHO guidelines are adhered to: a) botanical characteristics, sensory evaluation, foreign organic matter, microscopic, histological, histochemical assessment, quantitative measurements; b) physical and chemical identity, fingerprint chromatography, ash values, extractive values, moisture content, volatile oil and alkaloids tests, quantitative estimation protocols; c) estimation of biological activity, the values of bitterness, astringency hemolytic index, a factor swelling, heavy metals, microbial contamination as viable count total, pathogens like *E. coli*, *Salmonella*, *P. aeruginosa*, *S. aureus*, and *Enterobacteriaceae*; and e) microbial contamination and radioactive contamination [70].



A schematic representation of herbal drug standardization

6. Discussion

In contemporary medicine, medicinal plants have continued to play a significant role in the development of new drugs. A wide variety of complex bioactive metabolites of various chemical scaffolds can be found in abundance in plants. One helpful method for comprehending the phytochemical components of medicinal plants is the spectroscopic identification of their metabolites. [71] Without the need for complicated and time-consuming chromatographic purifications, metabolomics research aids in the simultaneous identification of many metabolites found in plants. One promising method for dereplicating metabolites found in extracts from therapeutic plants is metabolomics. [72–73] Based on the diagnostic FTIR peaks in the spectra, several phytochemical marker compounds, including liquirtin, piperine, gingerols, and curcumin, were found in the aforementioned study.

7. Conclusion

In this study we have investigated fingerprinting properties and metabolite profiling of the complex mixtures of the herbal samples like extracts and powders using FTIR based metabolomics approach. The characteristic FTIR spectra of some of the popular Indian medicinal plants were obtained. Various functional groups, such as -CHO, -COOH, -NO₂, -NH, and -OH were identified on basis of FTIR frequencies. The application of FTIR analyses was successful in analysis and identification of various secondary metabolites present in the selected medicinal plants. FTIR was found to be a simple, easy to use, rapid and inexpensive method for identification and detection of adulteration and for checking in any variation in herbal raw material. So it can be a very useful and supportive tool in the quality control and standardization of the herbal raw materials that are useful for phytopharmaceuticals and nutraceuticals industries.

REFERENCES

- [1] Peter AGM & De Smet, Herba remedies. *New Eng J Med*, 2002; 347: 2046-2056.
- [2] Waxler-Morrison, N. E., Plural medicine in India and Sri Lanka: do ayurvedic and Western medical practices differ? *Soc. Sci. Med*, 1988; 27: 531–544.
3. Gautam V, Raman RMV, Ashish K. Exporting Indian healthcare (Export potential of Ayurveda and Siddha products and services) Road beyond boundaries (The case of selected Indian healthcare systems). Gautam V, Raman RMV, Ashish K. (eds.) *Export-Import Bank of India*; Mumbai: 2003; p 4–54.
4. Sagar Bhanu PS, Zafar R, Panwar R. Herbal drug standardization. *The Indian Pharmacist* 2005; 4(35):19-22.
5. Amit J, Sunil C, Vimal K, Anupam P. Phytosomes: A revolution in herbal drugs. *The Pharma Review* 2007; 11: 13.
6. <http://www.who.int/research/en>.
7. Patel PM, Patel NM, Goyal RK. Quality control of herbal products. *The Indian Pharmacist* 2006; 5(45):26-30.
8. Vaidya ADB, Devasagayam TPA. Current status of herbal drugs in India: An overview. *J Clin Biochem* 2007; 41(1):1–11.
9. <http://www.morethanvitamins.co.uk/herbal-remedies-c> 24.html.
10. Pferschy-Wenzig, E. M., & Bauer, R. (2015). The relevance of pharmacognosy in pharmacological research on herbal medicinal products. *Epilepsy & behavior*, 52, 344–362.
11. EMEA. Quality of Herbal Medicinal Products. Guidelines. European Agency for the Evaluation of Medicinal Products (EMA), London, 1998.
12. SA Chavhan, SA Shinde, HN Gupta, Natural bioenhancers, *International Journal of Pharmaceutical Science and Research*. 2018; 3(1):29-37.
13. Barreto FS, Sousa EO, Campos AR1, Costa JGM, Rodrigues FFG. Antibacterial Activity of *Lantana camara* Linn and *Lantana montevidensis* Brigg Extracts from Cariri-Ceara, Brazil. *J Young Pharm*. 2010; 2(1):42-4.
14. Drug interactions between herbal and prescription medicines. *Drug Safety*. 2003; 26 (15):1075-92.
15. Shinde VM, Dhalwal K, Potdar M, Mahadik KR. Application of quality control principles to herbal drugs. *Int J Phytomed* 2009; 1:4-8.
16. Mor RS, Bhardwaj A, Singh S, Sachdeva A. Productivity gains through standardization-of-work in a manufacturing company. *J Manuf Technol Manag* 2019; 30:899-919.
17. Zafar R, Panwar R, Sagar Bhanu PS. Herbal drug standardization: *The Indian Pharmacist* 2005; 4(36): 21-25.
18. Vaidya ADB, Devasagayam TPA. Current status of herbal drugs in India: An overview. *J Clin Biochem* 2007; 41(1):1–11.
19. Patra KC, Pareta SK, Harwansh RK, Jayaram Kumar K. Traditional approaches towards standardization of herbal medicines -A review. *J Pharm Sci Technol* 2010; 2 (11): 372–379.
20. Straus SE. Herbal remedies. *New Engl J Med* 2002; 347: 2046–2056.
21. Indian Herbal Pharmacopoeia, Indian Drug Manufacturers' Association, Mumbai, 2002.
22. Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1996.
23. Patel PM, Patel NM, Goyal RK. Quality control of herbal products. *The Indian Pharmacist* 2006; 5(45):26-30.
24. Bhutani KK. Herbal medicines enigma and a challenge for science and guidelines for new initiatives. *J Nat Prod* 2003; 19(1): 3-8.
25. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine. *Pure Appl Chem* 2008; 80(10):2195–2230.
26. Seitz U, Bonn G, Oefner P, Popp M. Isotachophoretic analysis of flavonoids and phenolcarboxylic acids of relevance to phytopharmaceutical industry. *J Chromatogr* 1991; 559, 499-504.
27. O'Shea TJ. Capillary electrophoresis/electrochemistry. *Curr Sep* 1995; 14(1): 18-23.
28. Svickova M, Havranek E, Novak V. Determination of heavy metals in samples of herbal drugs using differential pulse polarography. *J Pharm Biol* 1993; 42(2):68-70.
29. Sharma AK, Gaurav SS, Balkrishna A. A rapid and simple scheme for the standardization of polyherbal drugs. *Int J Green Pharm* 2009; 3:134-140
30. Satheesh Madhav NV, Kumud Upadhyaya, Bisht A. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. *Int J Pharm Pharmaceut Sci* 2011; 3:56-59.

31. Patwardhan B. A method of treating musculoskeletal disease and a novel composition there of. US Patent 5494668, 1996.
32. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. *J Pharmacog Res* 2011;3(1):49-56
33. Meena AK, Rao MM, Panda P, Kiran, Yadav A, Singh U, et al. Standardisation of ayurvedic polyherbal formulation, Pancasama Churna. *Int J Pharmacog Phytochem Res* 2010;2(1):11-14.
34. Sanjay J, Sweta S, Rakesh B, Praveen K. Standardization of 'Dashamularishta': A polyherbal formulation. *Pharmacog J* 2009;1(3):54-57.
35. Kumar T, Chandrashekar KS, Tripathi DK, Nagori K, Pure S, Agrawal S, et al. Standardization of "Gokshuradi Churna" An ayurvedic polyherbal formulation. *J Chem Pharm Res* 2011;3(3):742-749.
36. Kasthuri KT, Radha R, Jayshree N, Austin A, Shanthi P. Development of GC-MS for a poly herbal formulation MEGNI. *Int J Pharm Sci* 2010;2:81-83.
37. Meena R, Meena AK, Khan SA, Mageswari S. Standardization of Unani polyherbal drug - Jawarish-e Darchini. *J Pharm Res* 2010;7(3):11-12.
38. Rajani M, Kanaki NS .Phytochemical standardization of herbal drugs and polyherbal formulations. *Bioactive Molecules and Medicinal Plants*, (Ramawat KG, Mérillon JM (Eds.) Springer 2008; 349-369.
39. Ahmad I, Aqil F, Owais M. Turning medicinal plants into drugs. *Modern Phytomed* 2006; 384:67-72.
40. Shrikumar S, Maheshwari U, Sughanti A, Ravi TK .WHO guidelines for standardization of herbal drugs. *Pharminfo.net* 2006; 2: 78-81.
41. Chandrakant K., Dere Pravin J., Honde Bharat S.,Kothule Sachin1, Kote Amol P. An overview of supercritical fluid extraction for herbal drugs, *Pharmacologyonline* 2011; 2: 575-596.
42. VyasN , Khan MY, Panchal S, Butani A, Kumar V. Supercritical fluid technology-An unlimited frontier in herbal research. *Int J Pharm Sci Nanotechnol* 2009;1(4):303 307.
43. Bertucco A, Franceschin G. Supercritical fluid extraction of medicinal and aromatic plants: Fundamentals and applications. In: *Extraction technologies for medicinal and aromatic plants*. Handa SS, Khanuja SPS, Longo G, Rakesh DD (eds.). International Centre for Science and High Technology. Trieste, 2008.
44. Wang Y, Xi GS, Zheng YC, Miao FS. Microwave-assisted extraction of flavonoids from Chinese herb *Radix puerariae*. *J Med Plant Res* 2010; 4(4):304-308.
45. Be´atrice K, Philippe C. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurised solvent extraction. *Phytochem Anal* 2002; 13:105–113.
46. Vivekananda M, Yogesh M, Hemalatha S. Microwave assisted extraction -An innovative and promising extraction tool for medicinal plant research. *J Pharmacog Rev* 2007;1(1):78-84.
47. Wir-Ferenc A, Biziuk M .Solid phase extraction technique– Trends, opportunities and applications. *J Environ Studies* 2006; 15(5): 677-690.
- [48] Zafar R, Panwar R, Sagar Bhanu PS. Herbal drug standardization: The Indian Pharmacist 2005; 4(36): 21-25.
- [49] Patra KC, Pareta SK, Harwansh RK, Jayaram Kumar K .Traditional approaches towards standardization of herbal medicines -A review. *J Pharm Sci Technol* 2010; 2 (11): 372 379.
- [50] Patel PM, Patel NM, Goyal RK. Quality control of herbal products. *The Indian Pharmacist* 2006; 5(45):26-30.
- [51] Indian Herbal Pharmacopoeia, Indian Drug Manufacturers' Association, Mumbai, 2002.
- [52] British Herbal Pharmacopoeia, British Herbal Medicine Association, 1996.
- [53] Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1996.
- [54] Bhutani KK. Herbal medicines enigma and a challenge for science and guidelines for new initiatives. *J Nat Prod* 2003;19(1): 3-8.
- [55] Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine. *Pure Appl Chem* 2008; 80(10):2195–2230.
- [56] Seitz U, Bonn G, Oefner P, Popp M. Isotachophoretic analysis of flavonoids and phenolcarboxylic acids of relevance to phytopharmaceutical industry. *J Chromatogr* 1991;559, 499-504.
- [57] O'Shea TJ .Capillary electrophoresis/electrochemistry. *Curr Sep* 1995;14(1): 18-23.
- [58] Svicekova M, Havranek E, Novak V. Determination of heavy metals in samples of herbal drugs using differential pulse polarography. *J Pharm Biol* 1993; 42(2):68-70.

- [59] Bauer R. Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standard can be achieved?. *J Drug Inform* 1998; 32:101-110.
- [60] Sharma AK, Gaurav SS, Balkrishna A. A rapid and simple scheme for the standardization of polyherbal drugs. *Int J Green Pharm* 2009; 3:134-140
- [61] Satheesh Madhav NV, Kumud Upadhyaya, Bisht A. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. *Int J Pharm Pharmaceut Sci* 2011;3:56-59.
- [62] Patwardhan B. A method of treating musculoskeletal disease and a novel composition there of. US Patent 5494668, 1996.
- [63] Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. *J Pharmacog Res* 2011;3(1):49-56
- [64] Shikha S, Mishra N. Genetic markers - a cutting-edge technology in herbal drug research. *J Chem Pharm Res* 2009; 1:1-18.
- [65] Lazarowych NJ, Pekos P. The use of fingerprint and marker compounds for identification and standardization of botanical drugs. *J Drug Inform* 1998; 32:497-512.
- [66] Kalpana J, Preeti C, Dnyaneshwar W, Bhushan P. Molecular markers in herbal drug technology. *Curr Sci* 2004; 87:159 165.
- [67] Jayvant K, Vaibhav S, Abhay H. Application of ISSR marker in pharmacognosy. *Curr Sci* 2009; 3: 216-228.
- [68] Usha K, Salim K, Mirza KJ, Mauji R, Abdin MZ. SCAR markers: A potential tool for authentication of herbal drugs. *Fitoterapia* 2010; 81:969-976.
- [69] Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. *J Nucleic Acids Res*. 1990; 18:6531–6535.
- [70] Shrikumar S, Maheshwari U, Sughanti A, Ravi TK .WHO guidelines for standardization of herbal drugs. *Pharminfo.net* 2006; 2: 78-81.
- [71] Dhakal S, Schmidt WF, Kim M, Tang X, Peng Y, Chao K. Detection of additives and chemical contaminants in turmeric powder using FT-IR spectroscopy. *Foods*. 2019;8(5):143. doi: 10.3390/foods8050143.
- [72] Jayasundar R, Ghatak S, Makhdoomi MA, Luthra K, Singh A, Velpandian T. Challenges in integrating component level technology and system level information from Ayurveda: insights from NMR phytometabolomics and anti-HIV potential of select Ayurvedic medicinal plants. *J Ayurveda Integr Med*. 2019;10(2):94-101. doi: 10.1016/j.jaim.2017.06.002, PMID 29306573.
- [73] Salem MA, DeSouza LP, Serag A, Fernie AR, Farag MA, Ezzat SM, et al. Metabolomics in the context of plant natural products research: From sample preparation to metabolite analysis. *Metabolites*. 2020;10:37.