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# Standardization, evaluation and quantification of Herbal drugs by various analytical methods

**Review Article**

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## Abstract

This review article encompasses the various evaluation parameters of standardization of different medicinal plants and its constituents. In today's life, more and more people of the world are turning towards the use of natural or herbal products as these have very less or negligible side effects. As the use of herbal medicinal products is increasing day by day, the questions regarding their quality are also arising. This is due to lack of parameters for the standardization of these products. There is a need to set different parameters or methods that can help to standardize the herbal medicinal plant materials. Various methods like physical, chemical, biological is used for standardization and quality evaluation of herbal medicinal plants. These methods can act as a basic tool for the quality evaluation of herbal plant materials. Different parameters of standardization are the fundamental tool for evaluating and assuring the quality of the herbal plant material and its products. This review article includes the quantitative evaluation of more than 20 herbal drugs. The evaluation parameters such as TLC, HPLC, HPTLC, GC, LC-MS, UPLC, UHPLC, UPLC-MS, UHPLC-MS will help to maintain quality of different herbal medicinal drugs as well as its formulations. The set parameters can ultimately lead to the quality and efficacy of the herbal medicinal formulations. Analytical methods and standardization can ensure the quality and consistency of active ingredients in herbal medicinal formulations.

**Key Words:** Herbs, Herbal medicine, Standardization, Marker, Chromatography, Validation, Evaluation parameters.

## Introduction

The term "HERBAL DRUGS" is referred as the means of plant or part of plants that have been transformed into phyto-pharmaceuticals by various processes which include collection or harvesting, drying and storage. Herbal medicine is a centuries-old method of healthcare that has been practised by people of all cultures throughout history. Ancient humans recognised their reliance on nature for a healthy life, and humankind has relied on a variety of plant resources for food, shelter, clothing, and medicine to treat a wide range of diseases since that time. Mesopotamian clay tablet writing and Egyptian papyrus are the first recorded documents describing the use of plants in healing. Primitive men and women were guided by nature, taste, and experience to cure illness by using plant, animal, and mineral parts that were not normally consumed. Primitive people learnt to distinguish favourable plants with good effects from those that were harmful by trial and error. (1-2)

Medicinal plants are considered as the source of medicine in practically all societies from the dawn of humanity. The prevalence of natural goods with therapeutic characteristics has been related to the widespread use of herbal treatments and healthcare formulation, such as those mentioned in ancient literatures such as the Vedas and are obtained from common traditional herbs and medicinal plants. The use of medicinal plants since Paleolithic age is carried out and can be estimated by the archaeological evidences. Till today, Herbal medicine is the primary source of health care for around 75-80% people of the world, primarily in poorer nations. Herbal medicines have seen a resurgence of interest in current years, as more and more people all over the world have begun to make use of medicinal or herbal plant-based products in their healthcare systems. Herbal medicine sales have plateaued to the point where these goods are now

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available to consumers as positive healthcare, similar to vitamins. As over-the-counter drug products, they can now be obtained in supermarkets, pharmacies, and a variety of other mainstream retail stores. This is due to the widespread perception that herbal medicines have no side effects and are inexpensive and readily available. (3-5)

Chemical drug side effects, scepticism of allopathic medicine's methodologies and assumptions, rising costs, and improved public access to knowledge on the safety and usefulness of medicinal plants have all contributed to a surge in interest in medicinal plants. When people try home remedies for acute, self-limiting diseases such as a cold, throat infection, or bee sting, it's typically because expert help is unreachable immediately, or it's too inconvenient, costly, or time taking. Other cultural factors, such as the environment and culture, and also "man-earth interaction," encourage the use of botanicals in rural areas. People seem to believe that if a region supports plants that can cure a disease, it will also support plants that can cure the disease. Large swaths of India's rural population lack access to modern medicine. Hundreds of primary health care centres, which are supposed to serve rural communities, are short on staff, diagnostic equipment, and medicine supplies. As a result, traditional medicinal systems are primarily reliant on the rural population. (5)

Herbs, herbal constituents, herbal preparations, and completed herbal products are all examples of herbal remedies. As a matter of tradition, herbal medications in some areas may contain natural organic or inorganic active compounds that are not of plant origin (e.g. animal and mineral materials). Herbs are whole plants, fragmented or powdered plant parts, such as seed, leave, bark, stem, flower, fruit, root, rhizome, or other plant parts. Herbal materials include fresh juices, gums, various oils such as fixed oils and essential oils, resins, and dried powdered herbs. In some countries, these materials may be prepared using a variety of local methods, such as roasting, steaming, or stir baking with honey, alcoholic beverages, or other ingredients. Herbal preparations, which can include powdered herbal ingredients as well as extracts, tinctures, and fatty oils, serve as the basis for finished herbal medicines. Herbal medicinal products are prepared through extraction, fractionation, purification, concentration, and other physical or biological procedures. Formulations made by steeping or heating herbal ingredients in alcoholic beverages, honey, or other ingredients are also included. Herbal preparations made from only one or more herbs are referred to as finished herbal products. The term "mixture herbal product" might be used if more than one herb is used. Excipients, in addition to active ingredients, may be present in finished herbal products and combination herbal products. However, finished products or herbal mixtures containing chemically defined active components, such as synthetic compounds and/or separated herbal ingredients, are not considered herbal. Traditional Medicine practises and therapies such as Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy, and Homeopathy make extensive use of herbal medications. (6-7)

# Methodology

## Herbal Drugs Standardization

Because of effectiveness and therapeutic nature for chronic disease with less toxicity, traditional medicine has grown in popularity around the world. Herbal medications are difficult to create because a number of factors influence biological efficacy and repeatable therapeutic effects. Standardization of herbal formulations standardization is necessary for evaluating quality pharmaceuticals based on active phytoconstituents concentrations, physical, chemical, phytochemical and in-vitro and in-vivo criteria. The three key criteria are necessary for the quality control of the herbal drugs viz. Authenticity, Purity and Assay. The evaluation of herbal formulation's quality is critical in order to give justification for their acceptance in the modern medical system. The lack of stringent quality control parameters for herbal ingredients and formulations is one of the primary issues confronting the herbal sector. The task of establishing a quality control standard for herbal crude drugs and their formulation entails biological evaluation of a specific disease region, chemical profile of the material, and the establishment of a final product specification. As a result, in the case of herbal drugs and products, the term "standardization" should refer to the full field of study, from medicinal plant cultivation through clinical use. Plant material and herbal treatments generated from it account for a significant share of the global market, thus internationally recognised quality control norms are required. Quality control of plant products is ensured by WHO through the use of current technology and the use of appropriate norms and standards. Other quality control measures must be investigated in order to overcome certain inherent shortcomings of the Pharmacopeial monograph. (8-10)

Herbal drugs standardization is a process by which a set of standard parameters for the characterization of the herbal drugs is specified. Because of the complex nature of plants-based drugs, the definite or reliable qualitative as well as quantitative characterization of herbal drugs is required. All features that can contribute to the quality of medicinal herbs, such as correct sample identity, organoleptic evaluation, pharmacogenetic evaluation, volatile matter, quantitative assessment such as ash values, extractive values, phytochemical analysis, test for the presence of foreign matter, microbial content, toxicity testing, and biological or therapeutic action, should be considered in standardisation methods. The phytochemical profile is especially important because it directly influences the activity of herbal medications. The use of fingerprinting profiles serves as a principle for assuring the quality of the drug's phytochemical profile, while quantification or expression of the marker components would be an additional criterion for determining the quality of sample. The term "phytochemical standardisation" refers to the collection of all information about the chemical ingredients found in herbal medicines.

Hence, for the standardization, the phytochemical assessment comprises of the following:

- Initial screening for the presence of various chemical groups.
- Quantification of chemical groups of interest (e.g., total alkaloids, total phenols, total triterpenoids, total tannins). Fingerprinting profiles are created.
- Fingerprinting profiles based on many markers.
- Quantification of key chemical components. (11-13)

**Need of Standardization**

As whole world is going towards the herbal plant-based products because of its high therapeutic value and lesser side effects. There is a need of various standardization parameters for standardization of herbal drugs as well as herbal-based products, so as to retard the chances of adulteration and to get the highly effective herbal drug product. Modern analytical procedures for the examination of herbal medications are critical for Ayurveda and traditional medicines to gain worldwide acceptability. A full and comprehensive pharmacogenetic assessment can provide scientific foundations for the high quality of traditional herbal remedies and ayurvedic products. The data obtained from macroscopic and microscopic examinations can serve as an extra benefit for identifying adulterants and authenticating genuine herbal materials, preventing genuine herbal materials from becoming contaminated. Furthermore, it will be beneficial for confirming criteria for standardization and identification of secondary metabolites such as alkaloids, tannins, glycosides, saponins and flavonoids. The microscopic and macroscopic investigations, identifications, physicochemical properties, pharmacogenetic parameters, and other parameters reported for the first time, in accordance with the process for formulation of standard herbal drugs in the pharmacopoeia and other standard texts, can play an important role for authenticating the herbs for future studies. The standardization parameters will able to assure the quality, safety and efficacy of the herbal drugs or the herbal products. (14-15)

Due to lack of quality control, severe consequences ranging from hepatotoxicity to death have occurred. As a result, instruments for identifying identification, purity, and quality are required for herbal substances, and these techniques must be technically adequate, quick, and cost-effective in order to meet GMP criteria. The World Health Organization has established standard criterion for evaluating quality, safety and efficacy of herbal drugs. Standardization of herbal drugs is difficult since many factors impact bio efficacy and repeatable therapeutic effect. To achieve a quality herbal product, accurate identification of plants, season, collecting area, extraction and purification, and rationalising the combination in the case of poly herbal medications should all be taken into consideration. (8)

**Role of Markers**

**Markers**

Marker compounds are naturally occurring constituents in a material that have been chosen for special attention (for identification or standardization purposes). Markers are chemically defined components of herbal medications that are of interest for quality control purposes regardless of therapeutic efficacy. Markers might be used to calculate the quantity of active ingredient in an herbal medication. Markers are those compounds which are pure and isolated secondary metabolites which comprises of terpenes, steroids, alkaloids, flavonoids and glycosides having various functional groups widely used for single or crude drugs. The use of unique markers that can be evaluated to identify across various kinds remains a favoured choice for quantitative investigations.

**Types of Markers**

1. Molecular markers or DNA markers
2. Chemical markers
   - A. Active principles
     - a. Active markers
     - b. Analytical markers
   - B. Negative markers
3. Biochemical markers

**Analytical evaluation technique in Herbal Drugs**

**TLC/HPTLC**

Prior to the development of instrumental chromatography methods such as GC and HPLC, TLC was the most widely used and versatile approach for herbal analysis established. Thin-layer chromatography is a technique for distributing a solute between two phases: a stationary phase that acts through adsorption and a mobile phase that takes the form of a liquid. HPTLC is a sophisticated instrumental approach that utilises all of TLC's capabilities. It is the most adaptable, dependable, and cost-effective separation method. The simplicity, variety, high velocity, particular sensitivity, and ease of sample preparation are all advantages of employing TLC/HPTLC to generate herbal medicine fingerprints. As a result, TLC is a useful tool for detecting the quality of herbal items and the possibility of adulteration. (17-19)

**GC–MS and herbal drugs**

The benefits of GC analysis of volatile oils are numerous. To begin, the volatile oil's GC provides a decent "fingerprint" that may be used to identify it. The composition and relative concentration of organic components in the volatile oil are unique to that plant, and the presence of impurities in the volatile oil can be easily detected.

Second, the volatile oil extraction is quite simple and can be standardised, and the components can be easily identified by GC–MS analysis. The great sensitivity of detection for practically all volatile chemical substances is definitely one of GC's advantages. This is especially true for FID detection and GC–MS analysis. (20-29)

The employment of a hyphenated GC–MS instrument, in particular, provides trustworthy information on the identity of the compounds. Advantages of GC–MS include: (1) with the capillary column, GC–MS has excellent separation ability and

can produce a high-quality chemical fingerprint; (2) With the mass linked the qualitative and relatively quantitative aspects of spectroscopy and the related mass spectral database. Information on the herb's composition could be obtained. GC–MS provides information that will be incredibly valuable for the need for more research to clarify the relationship herbal medicine's chemical ingredients and its efficacy Further research on pharmacology is needed. (30-33)

**High - Performance liquid chromatography and HPLC– DAD, HPLC–MS and others**

HPLC is a widely used technology for analysing medicinal herbs since it is simple to learn and use and is not limited by the volatility or stability of the sample. Reversed-phase (RP) columns are the most commonly used columns in the analytical separation of herbal medicines. HPLC–DAD is now used in almost all analytical laboratories throughout the world. The qualitative examination of complicated samples in herbal medicines becomes considerably easier than before with the addition of UV spectral information. The use of LC–MS and HPLC–DAD in the analysis of herbal medicines has clearly increased in recent decades. Several good reviews on the investigation of bioactive chemical components in plants and herbal medicines have been published, with HPLC, specially hyphenated HPLC techniques, being the most commonly used approach. Thanks to hyphenation techniques, which allowed one to identify the chromatographic peaks directly on-line by correlation with reported literature or with standard compounds, in most cases the LC–DAD–MS became a successful way for the rapid identification of constituents in herbals, and it can be used to avoid the time-consuming isolation of all compounds to be identified. (34-37)

**Table 1. Quantitative applications of HPLC method for identification of herbal drugs**

| Sr. No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | *Cassia angustifolia* Vahl (HPLC) | **Stationary Phase:** $C_{18}$ column<br>**Mobile Phase:** Methanol: Water: Acetic acid: tetrahydrofuran (60:38:2:2)<br>**Column:** Reversed phase µ- Bondapak $C_{18}$<br>**Retention Time:**<br>· Sennoside A: 9.88 min<br>· Sennoside B: 7.22 min | Separation and determination of Sennoside A and Sennoside B | (38) |
| 2 | *Paronychia argentea* Lam (HPLC) | **Stationary Phase:** ODS-3 Column<br>**Mobile Phase:** Distilled Water: Methanol: Acetic acid<br>**Column:** ODS-3(150 × 4.6) mm, 5 µm<br>**Retention Time:**<br>· Std. Luteolin: 1.3 min<br>· Std. Vanillic acid: 5.7 min<br>· Sample Luteolin: 1.6 min<br>· Sample Vanillic acid: 5.7 min | A. Optimization of Chromatographic Conditions<br>B. Determination of major components in Paronychia Argentea Lam dry extract<br>C. Method Validation | (39) |
| 3 | Salvia extracts (HPLC) | **Stationary Phase:** 515 HPLC Pump, waters 2487 dual wavelength absorbance detector & Millenium Software<br>**Mobile Phase:** Acetonitrile HPLC-Grade solvent<br>**Column:** Nova-Pak $C_{18,}$ 3.9 × 150 mm<br>**Retention Time:** 9.1 min | Determination of Rosmarinic acid | (40) |
| 4 | *Convolvulus pluricaulis* Forssk. (HPLC) | **Stationary Phase:** HPLC, Autosampler, UV Detector<br>**Mobile Phase:** Isocratic mixture of methanol and water containing 0.1% v/v formic acid (30:70)<br>**Column:** Phenomenex $C_{18}$(250mm × 4.6 mm,5 µm)<br>**Retention Time:**19.579 min | A. Validation of HPLC Method<br>B. Scopoletin Content<br>a. Hydroalcoholic Extract: 0.1738%<br>b. Methanol Extract: 0.0932%<br>c. Water Extract:0.0435% | (41) |

| | | | | |
|---|---|---|---|---|
| 5 | Brown marine macro algae (HPLC) | **Stationary Phase:** HPLC System equipped with degasser DGU-20A 5R, low-pressure quaternary pump LC20 and photo diode array detector SPD-M20<br>**Mobile Phase:** Water: Acetonitrile (5:3:2) containing 0.2% triethylamine<br>**Column:** Reverse phase Nova-Pak C-18 column (4 µm × 4.6mm × 250mm)<br>**Retention Time:** 15 min | Quantitative analysis of phenolics:<br>*1. S. cinereum*<br>a. Gallic acid:0.1144± 0.0096mg/g<br>b. p-hydroxybenzoic acid:0.0474±0.0005mg/g<br>*2. S. ilicifolium*<br>a. p-hydroxybenzoic acid:0.0094±0.0005mg/g<br>*3. S. tenerrimum*<br>a. Gallic acid:0.1165±0.0010mg/g<br>b. p-hydroxybenzoic acid:0.0186± 0.0005mg/g<br>*4. S. wightii*<br>Gallic acid:<br>0.0539± 0.0028mg/g<br>Vanillic acid, p-coumaric acid and ferullic acid = Absent in all | (42) |

**Table 2. Quantitative applications of HPTLC method for identification of herbal drugs**

| Sr. No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | *Oroxylum indicum* Vent. | **Stationary Phase:** Alumina Plate pre-coated with Silica Gel 60$F_{254}$<br>**Mobile Phase:** Chloroform: Methanol: Formic acid (8.8:0.7:0.5)<br>**Column:** CAMAG twin- trough chamber<br>**Retention Time:**20 min | **Crysin Content:**<br>Geographical Region<br>A. UP<br>a. Root:0.011%<br>b. Stem:0.002%<br>c. Leaf:0.006%<br>B. Western Ghats:<br>a. Root:0.014%<br>b. Stem:0.004%<br>c. Leaf:0.007% | (43) |
| 2 | *Bridelia montana* (Roxb.) Willd. | **Stationary Phase:** Alumina Plate pre-coated with Silica Gel 60$F_{254}$<br>**Mobile Phase:** n-hexane: ethyl acetate (2:8)<br>**Column:** Twin trough glass chamber<br>**Retention Time:** 15 min | A. **Preliminary Phytochemical Screening:** presence of Alkaloid, Glycoside, Tannin, Resins & Phenolic compounds<br>B. **HPTLC:** 6 Polyvalent phytoconstituents and corresponding ascending order of Rf values starts from 0.01-1.00; Highest concentration at 0.95 (26.75%) | (44) |
| 3 | *Tylophora indica* (Burm f.) | **Stationary Phase:** Alumina Plate pre-coated with Silica Gel 60$F_{254}$<br>**Mobile Phase:**<br>1. Chloroform extract – Chloroform: Methanol: Ethyl acetate (90:5:5)<br>2. Methanol extract- Toluene: Chloroform: Ethyl acetate (5:90:5)<br>3. Petroleum extract- Hexane: Ethyl acetate (40:60)<br>**Column:** Twin trough glass chamber<br>**Retention Time:**20 min | **HPTLC Fingerprinting:**<br>Different solvent systems were tried by hit and trial method for extracts: Methanolic extract, Chloroform extract and Petroleum ether extract. Satisfactory result was obtained in solvent systems developed, for chloroform extract Chloroform: Methanol: Ethyl acetate (90:5:5) v/v, Methanol Extract-Toluene: Chloroform: Ethyl acetate (5:90:5) v/v and for Petroleum ether extract-Hexane: Ethyl acetate (40:60) v/v.<br>The solvent system tried for Pet.Ether extract 12 peaks is observed, in methanolic extract 12 peaks is obtained too. However, in Chloroform 8 peaks is observed. The RF values obtained are calculated through WINCATS HPTLC software supplied with the instrument. | (45) |

**Table 3. Quantitative applications of HPLC method with UV detector for identification of herbal drugs**

| Sr.No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | *Cannabis sativa* Linn. | **A) HPLC:**<br>**Stationary Phase:** LaChrom Elite System consists of LaChrom Elite L-2200 autosampler, LaChrom Elite L-2130 pump, LaChrom Elite L-2350 column oven and LaChrom Elite L-2420 UV-VIS detector<br>**Mobile Phase:** Gradient Elution with pure-water and Acetonitrile (both with 0.1% Formic acid)<br>**Column:** Phenomenex Kinetex XB-C18 column (150 × 4.6mm;2.6 µm)<br>**Retention Time:** 15min<br><br>B) **UHPLC:**<br>**Stationary Phase:** HITACHI ChromasterUltra UHPLC system consists of 6270 autosampler, a 6310-column oven, a 6170 binary pump and a 6430 Diode Array Detector<br>**Mobile Phase:** Gradient Elution with pure-water and Acetonitrile (both with 0.1% Formic acid)<br>**Column:** Phenomenex Kinetex XB-C18 column (150 × 2.1mm; 1.7 µm)<br>**Retention Time:** 15min | A) Chromatographic Analysis<br>B) Extraction<br>C) Method development and validation<br>D) Stability<br>E) Quantification<br>F) Loss on drying | (46) |
| 2 | *Cuscuta chinensis* Lam. | **Stationary Phase:** Waters Alliance system equipped with a vacuum degasser, quaternary detector<br>**Mobile Phase:** Linear gradient with O-phosphoric acid 0.25% - acetonitrile<br>**Column:** ACE $C_{18}$ (4.6 × 250mm, 5µm)<br>**Retention Time:** 42min | A) Determination of Flavonoids<br>B) Method development and Validation | (47) |
| 3 | *Panex notoginseng* (Burkill) | **Stationary Phase:** HPLC system consists of a quaternary solvent delivery system, a line degasser, an auto sampler, a column temperature controller and UV detector<br>**Mobile Phase:** A) Deionized water: Acetic acid (100:0.1v/v)<br>B) Acetonitrile: Acetic acid (100:0.1v/v)<br>**Column:** Ultimate™ XB-$C_{18}$ column (250mm× 4.6mm,5 µm)<br>**Retention Time:** 85min | A) Optimization of HPLC separation<br>B) HPLC-UV fingerprinting of the XST injection<br>C) Identification of characteristic peaks<br>D) Determination of main saponins in the XST injection | (48) |

**Table 4. Quantitative applications of LC-MS method for identification of herbal drugs**

| Sr. No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | *Curcuma aeruginosa* Roxb. | **Stationary Phase:** UPLC-MS equipped with a binary pump; LC connected to QTOF mass spectrometer coupled to ESI. MS was Xevo G2-S QTOF with positive ionisation mode<br>**Mobile Phase:** A)5mM ammonium formic<br>B)0.1% formic acid in acetonitrile<br>**Column:** Acquity UPLC HSS $C_{18}$ (2.1 × 150mm,1.8 µm)<br>**Retention Time:** 15min | A) Biological activity of C. aeruginosa<br>B) Identification of Bioactive components of C. aeruginosa rhizome by LC-MS<br>C) Correlation between metabolites composition and antioxidant activity<br>D) Correlation between metabolites composition and toxicity | (49) |
| 2 | *Tribulus terrestris* Linn. | **Stationary Phase:** UHPLC Flexer FX-10 and a QTOF 4600<br>**Mobile Phase:** A)70% 1mM ammonium acetate buffer<br>B)30% acetonitrile<br>**Column:** Phenomenex Luna $C_{18}$ (4.6 × 100mm,3 µm)<br>**Retention Time:**10min for samples; 3min for standard | A) LC-MS/ MS analysis<br>B) Method development and validation for protodioscin | (50) |
| 3 | *Panax notoginseng* (Burkill) | **Stationary Phase:** HPLC-MS; Agilent 1100 series LC system equipped with a binary solvent delivery system, an auto sampler, a column temperature controller, a photo diode array detector and a Finnigan LCQ Deca $XP^{plus}$ ion trap mass spectrometer via ESI interface<br>**Mobile Phase:** A) Deionized water: Acetic acid (100:0.1v/v)<br>B) Acetonitrile: Acetic acid (100:0.1v/v)<br>**Column:** Ultimate™ XB-$C_{18}$ column (250mm × 4.6mm,5 µm)<br>**Retention Time:** 85min | A) Optimization of HPLC separation<br>B) HPLC-UV fingerprinting of the XST injection<br>C) Identification of characteristic peaks<br>D) Determination of main saponins in the XST injection | (48) |
| 4 | *Carica papaya* Linn. | **Stationary Phase:** LC-MS/MS equipment [UHPLC (Thermo Scientific ACCELLA type 1250)]<br>**Mobile Phase:** A)0.1% sulphuric acid in distilled water<br>B)0.1%formic acid in acetonitrile<br>**Column:** Hypersil Gold (50mm × 2.1mm × 1.9mm)<br>**Retention Time:** 4min | A) Carpaine analysis with LC-MS | (51) |
| 5 | *Asparagus racemosus* Wild. | **Stationary phase:** hplc-q-tof-ms/ms<br>**Mobile phase:** a)0.1% formic acid in water<br>B)0.1%formic acid in acetonitrile<br>**Column:** phenomenex luna $c_8$ column (150 × 4.6mm,5 µm)<br>**Retention time:** 12min | A) Saponin analysis by HPLC-Q-TOF-MS/MS<br>B) Method validation | (52) |
| 6 | *Ascophyllum nodosum* Linn. | **Stationary Phase:** LC-MS/MS performed on Sciex API 4000 Triple Quadrapole Mass Spectrometer coupled to an Agilent 1100 HPLC<br>**Mobile Phase:** A)0.01% formic acid and 5mM ammonium formate<br>B)95%acetonitrile / 5% water<br>**Column:** Alltima HP HILIC Expedite™ MS column (4.6 × 20mm,1.5 µm)<br>**Retention Time:** 15min | A) LC-MS/MS analysis of betaine content<br>B) Optimization of betaine extraction conditions | (53) |

**Table 5. Quantitative applications of UPLC method for identification of herbal drugs**

| Sr.No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | Resina Draconis a type of dragon's blood resin obtained from *Dracaena cochinchinensis* (Lour.). | **A) UPLC-PDA**<br>**Stationary Phase:** UPLC System with auto sampler and photo diode array (PDA) detector<br>**Mobile Phase:** Linear gradient of water and acetonitrile<br>**Column:** HSS $C_{18}$ column (2.1mm × 100mm, 1.8µm)<br>**Retention Time:** 30min<br><br>**B) HPLC-DAD**<br>**Stationary Phase:** Agilent 1100 series HPLC-diode array detector (DAD)<br>**Mobile Phase:** Gradient of water and acetonitrile<br>**Column:** Alltima $C_{18}$ column (4.6mm × 250mm,5 µm)<br>**Retention Time:** 50min | **Optimization of the preparation methods for the sample solution:** Comparison of preparation methods of sample solution by comparing chromatograms obtained from various extraction solvents | (54) |

**Table 6. Quantitative applications of UPLC-MS method for identification of herbal drugs**

| Sr.No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | *Ambrosia maritima* Linn. *And Ammi majus* Linn. | **Stationary Phase:** Water's Acquity UPLC system equipped with a binary solvent delivery system, an autosampler, a column manager and a tunable MS detector<br>**Mobile Phase:** A) Acetonitrile<br>B) 0.5% formic acid in water<br>**Column:** Monolithic capillary silica based $C_{18}$ column<br>**Retention Time:** 20min | A) Antioxidant activity of the extracts and fractions<br>B) Fingerprinting of active fractions using HPTLC<br>C) Quantification of gallic and ellagic acid content in the fractions | (55) |
| 2 | *Khaya senegalensis* (A.Juss.) | **Stationary Phase:** Acquity UPLC system<br>**Mobile Phase:**<br>1) Isocratic 95% Water: Formic acid (99.9:0.1 v/v) and 5% Acetonitrile: Formic acid (99.9:0.1 v/v)<br>2) Ammonium acetate 50mM buffer adjusted to pH 5 and 100% Acetonitrile<br>**Column:** HSS T3 column (100 × 1.0mm,1.8 µm)<br>**Retention Time:** | A) UPLC of acetone extract<br>B) Antimicrobial effect of acetone extract<br>C) Cytotoxicity of acetone extract<br>Identification of five flavonoid glycosides and twelve limonoids | (56) |

**Table 7. Quantitative applications of UHPLC-MS for identification of herbal drugs**

| Sr. No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | Ginger | **Stationary Phase:** Waters Acquity instrument equipped with binary solvent delivery system, auto sampler, column manager and tunable mass spectroscopy detector<br>**Mobile Phase:** Degassed acetonitrile: water (9:1 v/v)<br>**Column:** BEH $C_{18}$ column (100mm × 2.1mm; 1.7 µm)<br>**Retention Time:**3min | A) Method Validation<br>B) Gingerol isomers were separated | (57) |

**Table 8. Quantitative applications of UHPLC method with PDA detector for identification of herbal drugs**

| Sr.No. | Name of Drug/ Chemical | Parameters for Validation | Evaluation Parameters of Standardization | References |
|---|---|---|---|---|
| 1 | *Withania somnifera* Linn. | **Stationary Phase:** Shimadzu Nexera X2 consisting of quaternary pump, auto sampler, column oven with diode array detector coupled with LCMS-8045<br>**Mobile Phase:** Gradient elution of water and acetonitrile<br>**Column:** Phenomenex Luna 5µm C8 (250mm × 4.6mm × 5µm)<br>**Retention Time:** | **A Novel UHPLC-PDA method for identification of 11 Withanosides and Withanolides**<br>A) Method optimization<br>B) Method validation<br>C) Mass spectroscopy<br>D) Profile pattern and multicomponent analysis calculations of *Withania somnifera* Linn root extract (WSE)<br>E) Preliminary comparison of quantification of the *Withania somnifera* Linn root extract (WSE) by External Standard method | (58) |

## Discussion

Evaluation parameters of standardization can contribute to the quality control of herbal product and ensures its activity and efficacy of phytoconstituents. The use of analytical procedures or methods will aid in enhancement of herbal drug products. For the rise of herbal market, there should be a globally acceptable set of standards for the quality control of herbal medicinal plant material as well as its formulation. The set of standards can support the quality and ultimately the efficacy of the herbal medicinal formulations.

The set of standards are fixed by using various techniques like TLC, HPTLC, HPLC, GC, UPLC and other hyphenated techniques like LC-MS, GC-MS, HPLC-MS, UPLC-MS, UHPLC-MS by using various detectors like UV detector, PDA detector, DAD, etc. These techniques are useful for detecting the quality of herbal drugs or its products. Analytical techniques are used for the identification and separation of various compounds of herbal plants. Simple chromatographic techniques are used to identify the various compounds like alkaloids, glycosides, tannins, sennosides, coumarins, flavonoids, isoflavonoids, etc. The hyphenated techniques are the combination of two techniques that are separation techniques and detection techniques. These techniques are helpful in demonstrating both qualitative and quantitative analysis of numerous components of the herbal drugs. Various hyphenated techniques are also useful in quality control and standardization of herbal crude drugs as well as herbal medicinal formulations by chemical fingerprinting analysis.

In HPLC method, various plant species like *Cassia Angustifolia, Paronychia Argentea* Lam, Salvia, *Convolvulus Pluricaulis*, Brown marine macro algae are used. This method has provided the different components present in these species. In HPTLC method, various plant species like O*roxylum indicum*, *Bridelia montana*, *Tylophora indica* are used for the Phytochemicals screening. In case of *Tylophora indica*, different solvent systems were tried by hit and trial methods to get a satisfactory resolution in solvent system.

In HPLC-UV method, various plant species like *Cannabis sativa* Linn., *Cuscuta Chinensis* Lam., *Panex Notoginseng* are used. This method has been proved helpful in determination of flavonoids in *Cuscuta Chinensis* and saponins in *Panex Notoginseng*; in fingerprinting of XST injection of *Panex Notoginseng*. In LC-MS method, *Curcuma Aeruginosa, Tribulus Terrestris* Linn., *Panex Notoginseng, Carica Papaya, Asparagus Racemosus, Ascophyllum Nodosum* are used. This technique is used for identification of bioactive component of *Curcuma Aeruginosa* as well as the co-relation between metabolites composition with anti-oxidant activity and toxicity. This technique is also used for carpaine, saponin and betaine analysis in *Carica Papaya, Asparagus Racemosus* and *Ascophyllum Nodosum* respectively.

The UPLC-PDA and UPLC-DAD method is applied on Resina Draconis to provide chemical fingerprinting analysis and validation study of this method. The UPLC-MS method is used for fingerprinting of active constituents and to determine the amount of gallic and ellagic acid in *Ambrosia Maritima* Linn. and *Ammi Majus* Linn. This method is also used to determine the antimicrobial effect and cytotoxicity of the acetone extract of *Khaya Senegalensis*. The UHPLC-MS method is demonstrated for study of method validation and to separate gingerol isomers present in Ginger. The UHPLC method with PDA detector is used for the identification of eleven withanosides and withanolides.

In this way, various types of chromatographic techniques are helpful in identification, quality control and standardization of numerous herbal medicinal plant species.

## Conclusion

There are numerous factors which affect the quality of herbal plant material or its products. The advancement in the analytical evaluation can lead to increase in the quality of herbal medicinal products. Standardization is the protocol for ensuring the quality and consistency of active principles of medicinally active plants. Thus, standardization will serve to set the quality control specifications, which can assist the worldwide expansion of herbal medicinal plants.

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