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Analytical Method Development for Quality Control and Standardization of Medicinal Plants: A Critical Review

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Abstract

The demand for herbal medicine is increasing as herbs are less toxic, affordable, and culturally acceptable than the conventional drugs in developing countries. Thus there increases the demand for standardization to ensure safety, purity and quality of medicinal plant. Standardization is a process of confirming herbal medicine identity and determination of its quality and purity. It involves botanical, physical, chemical and biological evaluations. These require application of different analytical methods and tools for standardization and phytochemical investigation of medicinal plants. The aim of this review is to explore analytical methods used for standardization of medicinal plants. A comprehensive web-based literature review is employed in order to retrieve relevant information using international scientific databases including PubMed, Science direct, Web of Science, Google scholar. The review of selected 34 original published research articles were evaluated and summarized. The result this review showed that several analytical methods are employed and developed for standardization including chromatographic: TLC, GC, HPTLC and HPLC fingerprints, spectroscopic; UV, Fluoerence, MS, FT-IR, AAS and NMR are commonly applied techniques for different analytical purposes which are mainly used in physicochemical and phytochemical characterization of medicinal plants. Gel electrophoresis and DNA fingerprint are also applicable for botanical identification. The choice of analytical methods may depend on phytochemical, physicochemical nature of medicinal plant, selectivity and accuracy of analytical method. Development of selective analytical method is recommended for pesticide and Alfa toxin analysis to further ensure safety, quality and for complete standardization of medicinal plants.

Keywords: Herbal medicine, standardization, phytochemical investigation, analytical methods, quality control, purity

INTRODUCTION

Traditional medicine refers to the use of indigenous medicinal and aromatic plants, animal parts, or organic and inorganic materials for preventive and therapeutic purposes [1]. Traditional medicines are medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant materials, or combinations thereof, whether in the crude state or as plant preparations [1].

Medicinal plants are in use for the purpose of treatment of different ailments and diseases since centuries. Over 80% of the world population depends on herbal medicines and product for healthy living [2]. According to WHO report, traditional medicine represents an important component part of health care provision in many African countries. In some countries, it has been estimated that up to 60–90% of the population relies on traditional medicines for their health care needs

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[3]. Traditional medicinal practices are also common in Ethiopia in which about 90% of the population in the country uses plant based traditional medicines as their major primary health care [3].

The reasons for increasing demands are medicinal plants often being more available, affordable, sometimes perceived as more effective than conventional drugs, culturally acceptable and their relatively lower cost. However, most of these medicinal plants which are claimed to be effective in traditional practice for various diseases lack scientific documented evidence of their safety, efficacy or quality [3].

This increases the need for standardization and quality control of medicinal plants. Standardization is the process of evaluation of the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and biological observations [4]. Standardization of the herbal drugs begins from the collection of the herbal drug to its packaging/use as medicine. The authenticity, quality and purity of herbal drugs are established by reference given in pharmacopoeia [5]. The pharmacopoeia prescribes (numerical value) like structural, analytical, physical standards for the drugs. The important standards mentioned in pharmacopoeia are shown in Figure 1 [5].

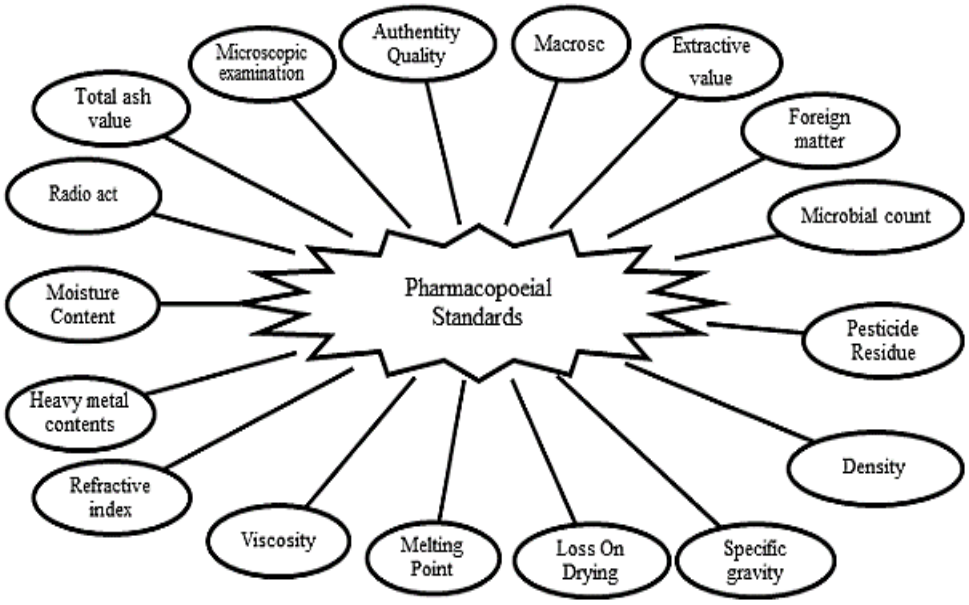


Figure 1. Standardization Parameter of Medicinal Plants.

According to WHO guidelines, standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion [6–8]. This includes the following basic evaluation for quality of herbal medicines [6, 7]: all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs [39].

The processes mentioned above involve wide range of scientific investigations, which include physical, chemical and biological evaluation employing various analytical methods and tools. The specific aims of such investigation in assuring herbal quality are as varied as the processes employed [6, 7].

Despite efforts by national authority and international organizations, there are several challenges for standardization of medicinal plants. These are [8]: Herbal drugs are usually mixtures of many constituents, the active principle(s) is (are), in most cases unknown, plant materials are chemically and naturally variable due to soil and environmental factors, chemo-varieties and chemo cultivars exist due to presence of adulterants, the source and quality of the raw material are variable and the methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents) also affect herbal quality [8]. Therefore, it is very important to employ selective and fast modern analytical approaches and methods to examine safety, purity and quality of medicinal plants for effective standardization.

OBJECTIVES

General Objective

The general objective of this review is to explore, synthesize and compile evidence of analytical methods used for the quality control and standardization of medicinal plants.

Specific Objectives

- To identify and describe analytical methods used for phytochemical analysis of medicinal plants.
- To identify and describe analytical methods used for physicochemical analysis of medicinal plant.
- To identify potential applications in standardization of medicinal plants.

METHODOLOGY

Search Strategies

A web-based research literature search strategy was employed for studies reported on quality control and standardization of medicinal plants with analytical methods commonly used for their investigations. Relevant literatures were gathered by two different search approaches, including: Search for published journal articles using international scientifc databases including PubMed, Science direct, Web of Science, Google scholar and using Google search engine as a general for supplementary guidelines and standards.

Literature search was performed using these key terms:

Standardization/medicinal plants, phytochemical investigation/medicinal plants, Analytical method/standardization, physicochemical analysis/medicinal plant

Inclusion and Exclusion Criteria

Published studies reporting on quality control and standardization medicinal plant are included for review. The following types of research data were excluded (Figure 2):

- Data from non-open access journal articles or partially accessed (abstract only) articles.
- Duplicates,
- Not related to study topic objectives (standardization of medicinal plants).
- Review articles.
- Unpublished research data.

Data Extraction and Evaluation

Analytical methods used for the phytochemical investigation and standardization of medicinal plants were entered in to excel spreadsheet and summarized using descriptive statistics (tables and charts). The review of the selected 34 original research articles was included for data evaluation and their detailed characteristics of the studies such as botanical name, part used, the extraction method, analytical method for phytochemicals analysis and physicochemical analysis, year of publication, were analyzed and presented using tables.

RESULTS

Data Evaluation for Analytical Methods

Results of data evaluation for analytical methods used are summarized as following (Table 1):

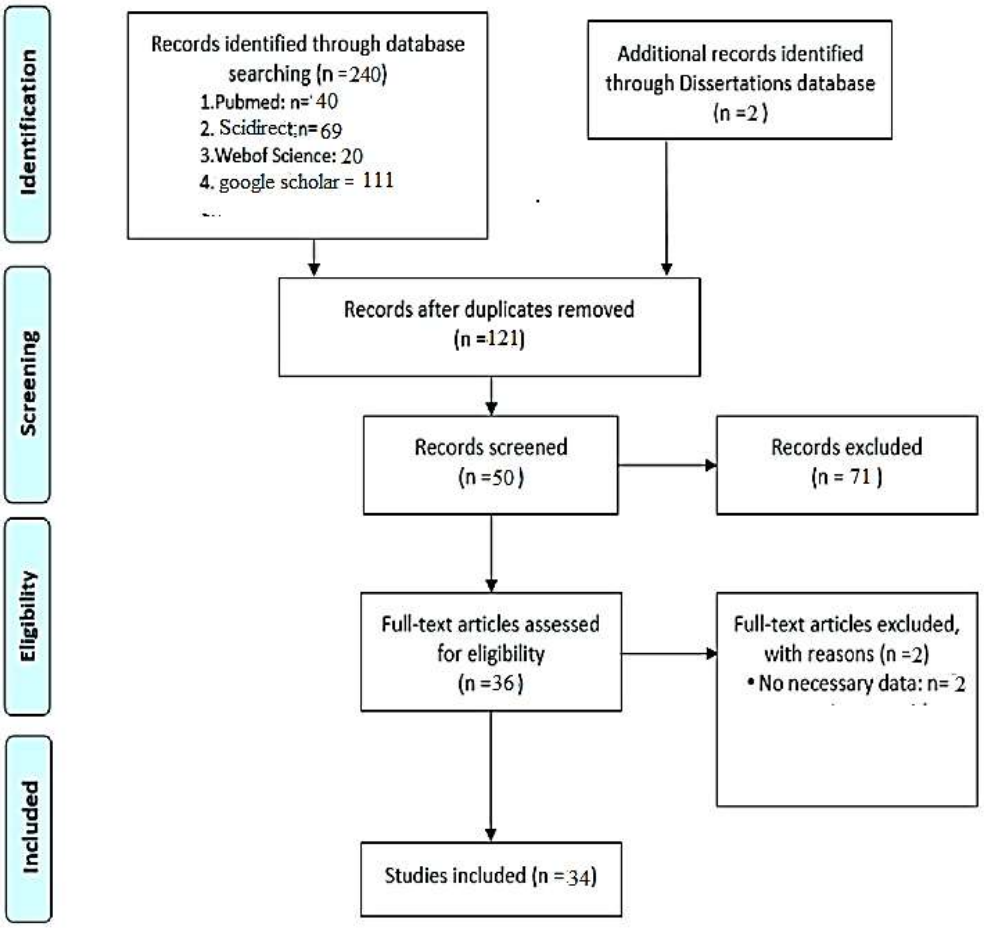


Figure 2. Flowchart for Selection of Relevant Literature for the Review.

Table 1. Phytochemical Analysis of Selected Medicinal Plants.

Scientific Name	Part Used	Publication Year	Analytical Method	Constituents	Reference
Piper longum Linn	Fruit	2006	RP-HPLC	sesamin, brachystamide guineensine	[20]
Woodfordia fruticosa	Flower	2014	TLC Fingerprint, Fluorescence	ellagic acid, gallic acid	[17]
Dioscorea bulbifera	Whole	2013	AAS, UV	Vit C, Vit D	[29]
Cucurbita maxima	Seed	2012	TLC, HPTLC, GC/MS	glucaosazone	[23]
Aerva lanata	Whole	2014	FT-IR		[28]
Curcuma caesia	Leaf	2017	GC MS, FT-IR	α-Santalol, Retinal, Ar-tumerone	[27]
Boswellia serrate	Leaf	2012	TLC, IR, GC/MS, NMR	Tetrahydro-2H-pyran-2, 3, 4, 5-tetrol	[30]
Aloe vera	Stem	2012	HPTLC Fingerprint	Berberine, gallic acid	[18]
Entada Africana	leaf $ stem	2019	UV-Vis, Fluorescence		[24]

Fluorescence Analysis of Medicinal Plants

Result of fluorescence analysis of medicinal plants is summarized as following (Table 2):

DISCUSSION

Based the above scientific studies, a different plant part is used for standardization and phytochemical investigations. The most frequently used parts are illustrated in Figure 3. For all these investigations

recommended standards are used for collection, drying, storage, transport and preparation of medicinal plants (Table 3) [9, 10, 2, 16, 17, 18, 20, 23, 24, 26–53].

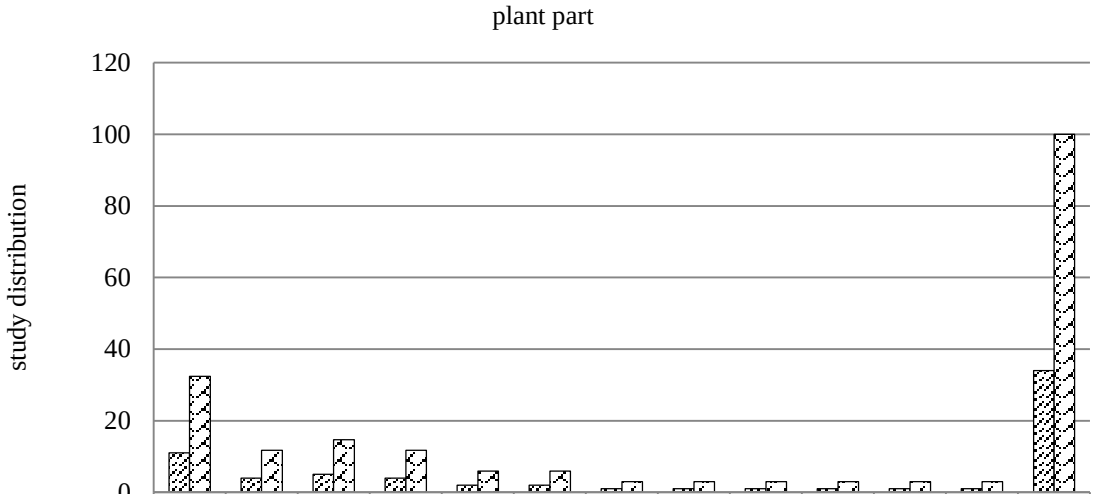
Table 2. Fluorescence Study of Selected Medicinal Plants.

Study	Scientific Name	Part Used	Reagent Used	Reference
Physicochemical properties	*Butea frondosa Koen.*	Leaf	H_2SO_4, HNO_3, NaOH, $FeCl_3$ and Picric acid	[31]
>>	*Woodfordia fruticosa*	Flower	NaOH, HCl and H_2SO_4	[17]
>>	*Cajanus scarabaeoides*	Whole	NaOH, HCl, H_2SO_4, HNO_3 and KOH	[26]

Table 3. Detail Characteristics of Studies Included (n=34) for Review.

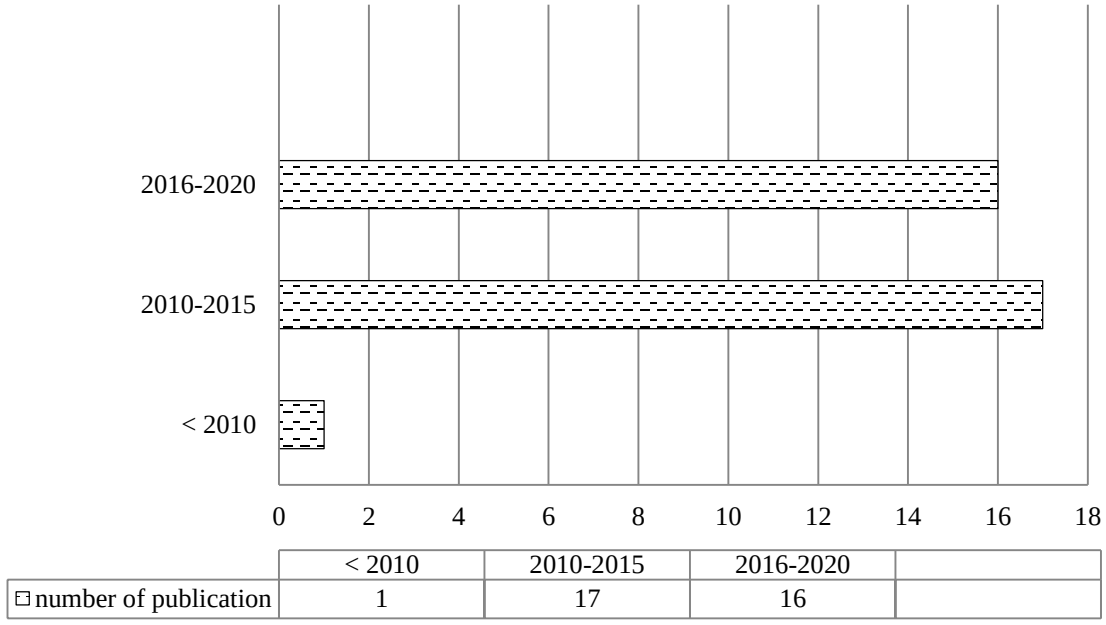
Scientific Name	Collection Site	Part Used	Extraction Method	Pysico-Method	Pyto-Method	Ref.$ Pub.y
Moringa Oleifera	Ethiopia	Leaf	Soxhlet	Standard	Standard Test	[33], 2014
Fadogia cienkowski	Nigeria	Leaf	Maceration	Standard	Standard Test	[34], 2019
Enicostemma Littorale	Sri Lanka	Whole	Soxhlet	Standard	Standard Test	[35], 2013
Caesalpinia crista L	India	Root	Soxhlet	Fluorescence	Standard Test	[36], 2019
Acacia auriculiformis A.	India	Stem Bark	Soxhlet	Standard	Standard Test	[37], 2017
Limonium stocksii	India	Leaf $ Stem	Solvents Extraction	Fluorescence	Standard Test	[38], 2018
Quercus infectoria	Sudan	Galls	Solvents Extraction	Standard	UV	[39], 2018
Amaranthus Spinosus /inn	India	Root	Soxhlet	Standard	TLC	[40], 2011
raetem (forssk) Webb	Libya	Leaf	Soxhlet	Not Specified	Standard Test	[41], 2017
Sesamum indicum L	India	Seed	Soxhlet	standard	Standard Test	[42], 2018
Woodfordia fruticosa	India	Flower	Soxhlet	Fluorescence	TLC Fingerprint	[17], 2014
Aerva lanata	India	Whole	Soxhlet	Not Specified	FT-IR	[28], 2014
Aloe vera	India	Leaf	Solvents Extraction	Not Specified	HPTLC	[18], 2012
Argemone mexicana	Mali	Leaf	Not Specified	Standard	Standard Test	[43], 2020
Dioscorea bulbifera	Malaysia	Whole	Not Specified	Standard	AAS	[29], 2013
Cassia fistula	India	Leaf	Solvents Extraction	Not Specified	HPTLC	[44], 2014
Cayratia trifolia	India	Stem	Not Specified	Fluorescence	Standard Test	[45], 2012
Cissampelos pareira	India	Stem	Solvents Extraction	Standard	TLC	[46], 2012
Crotalaria lachnosema	Nigeria	Leaf	Solvents Extraction	Standard	TLC	[47], 2012
Cucurbita maxima	India	Seed	Solvents Extraction	Standard	TLC, HPTLC, GC/MS	[23], 2012
Curcuma caesia	India	Rhizomes	Soxhlet	Not Specified	GC MS, FT-IR	[27], 2017
Lasia Spinosa	India	Whole	Percolation	Fluorescence	TLC	[16], 2013
Pterocarpus santalinus	India	Leaf $ Stem	Solvents Extraction	Fluorescence	Standard Test	[48], 2017
Strychnos nux	India	Seed	Solvents Extraction	Standard	HPTLC	[49], 2012
Butea frondosa Koen.	India	Leaf	Soxhlet	Fluorescence	TLC	[31], 2012
Boswellia serrata	India	Leaf	Soxhlet	Not Specified	TLC, IR, GC/MS, NMR	[30], 2012
Cajanus scarabaeoides	India	Whole	Maceration	Fluorescence	UV	[26], 2018
Cassia surattensis	India	Seed	Maceration	Standard	Standard Test	[2], 2020
Madhuca Indica	India	Leaf $ Stem	Not Specified	Fluorescence	Standard Test	[50], 2015
Mallotus rhamnifolius	India	Leaf	Soxhlet	Standard	Standard Test	[51], 2017
Calliandra calothyrsus Meissn	Indonesia	Leaf	Digestion	Standard	DPPH Assay	[52], 2019
Piper longum	India	Fruit	Solvents Extraction	Not Specified	RP-HPLC	[20], 2006
Lunasia amara	Indonesia	Wood	Solvents Extraction	Not Specified	UFLC	[53], 2016
Entada africana	Ghana	Leaf $ Stem	Not Specified	Fluorescence	UV	[24], 2019

Based on these studies, sample preparation involves extraction of crude drugs for further analysis using different solvents depending on nature of medicinal plant and solvent extraction capacity by different methods such as maceration, soxlet, percolation, digestion and continuous solvent extractions [9, 2, 16, 30, 49, 52].



	leaf	seed	whole	leaf $ stem	stem	root	stem bark	flower	gall	fruit	rhizome	wood	total
frequency	11	4	5	4	2	2	1	1	1	1	1	1	34
percent	32.35	11.76	14.71	11.76	5.882	5.882	2.941	2.941	2.941	2.941	2.941	2.941	100

Figure 3. Chart of Frequently Used Plant Parts (%) from (n=34) included in Review.



	< 2010	2010-2015	2016-2020	
number of publication	1	17	16	

Figure 4. Summary of Studies Included in the Review (n=34) by Year of Publication.

Phytochemicals are naturally occurring chemical compounds found in the medicinal plants, which serve for defense mechanism and prevention from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [9]. These secondary metabolites are responsible for medicinal activity of the plant [10]. Numbers of plants were screened for secondary metabolites for their medicinal values [10].

The medicinal plants occupy significant position in dominant sources of drug discovery;

standardization of a crude drug and correct identification of plant is crucial to maintain efficacy [11]. Standardization parameters include physicochemical, phytochemical, fluorescence analysis, macroscopic, microscopic and powder evaluation of the plant. Evaluating all these parameters will ensure and help in maintaining quality, purity and efficacy of the plant drug for its various uses. It will prevent the plant drug from adulteration and substitution intentionally or unintentionally [12].

Full investigation of phytochemicals in the medicinally important plants should be carried out, as this would be beneficial in standardization and dose determination of herbal drugs [13]. The process of standardization can be achieved by stepwise pharmacognostical, phyto- and physico-chemical studies [13]. Various advanced methods such as chromatographic, spectrophotometric and use of molecular biomarkers in fingerprints are employed in standardization of herbal drugs [13].

The preliminary phytochemical screening provides the qualitative analysis to explore the presence of important metabolites which inludes flavonoids, tannin carbohydrate, reducing sugars, antroquinones, steroids and phenoids, saponins, glycosides, alkaloids, proteins, free amino acids, oils and fats [14]. Standard chemical assay is commonly used for this purpose.

WHO recommended standard techniques are commonly employed method for determination of physicochemical parameters such as loss on drying, total ash, acid insoluble ash, water-soluble ash, sulphated ash and extractive values.

Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds [15, 16]. Analytical tools like Thin layer chromatography (TLC) and High performance thin layer chromatography (HPTLC) allow the detection, identification and estimation of chemical markers within the plant extract that can also be used for standardization of herbal formulations [15].

Thin layer chromatography (TLC) and HPLC are the most commonly used methods for obtaining chemical fingerprints and identification of the crude plant extracts. Chemical fingerprints through chromatographic techniques are more commonly used for standardization and are obtained in terms of one or more marker compounds [15].

TLC is used extensively in the phytochemical evaluation of herbal drugs because it enables rapid analysis of herbal extracts with minimum sample clean-up requirement, provides qualitative and semi-quantitative information of the resolved compounds [16]. In TLC fingerprinting, the data that can be recorded using a high performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, λ max and shoulder inflection/s of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample [16, 17]. Experimental investigation conducted using thin layer chromatography showed the presence of various tannins (gallic acid and ellagic acid), flavonoids (quercetin and myricetin) and sterols (betasitosterol) in the methanolic extract of *Woodfordia fruticosa* leaves [17].

High Performance Thin layer Chromatography is a modified version of thin layer chromatography. High Performance Thin layer Chromatography is planer chromatography where separation of sample components is done on high performance layers with detection and acquisition using an advanced work-station which increases efficiency of separation [18, 19]. HPTLC is suitable for qualitative, quantitative and micro-preparative chromatography. Experimental study of quantitave analysis through HPTLC revealed the presence of 2.74 and 0.543% of berbirene and gallic acid in Aloe vera extract [18].

HPTLC technique is widely employed in pharmaceutical industry in process development, identification and detection of adulterants in herbal product and it also helps in identification of pesticide

content, mycotoxins and in quality control of herbs and health foods [19].

HPLC method separates compounds on the basis of their interaction with solid particles of a tightly packed column and the solvent of the mobile phase. HPLC provides both quantitative and qualitative analysis in a single operation. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases [20]. In a recent study report, a reversed-phase high-performance liquid chromatography method was developed to quantify active principles in the plant material of *Piper longum*. As a result, sesamin was identified to be present in maximum quantities (0.91%) whereas brachystamide B was found in minimum quantity (0.01%) [20].

Gas chromatography is applicable for volatile compounds [21]. In this method, species distribute between a gas and a liquid phase. The gas phase is flowing and the liquid phase is stationary. When the sample molecules are in liquid phase they are stationary. The rate of migration depends on how much of chemical species is distributed into liquid phase. Higher the percentage of material in the gaseous state, faster will be the migration. The species which distributes itself 100% in the stationary state will not migrate. If a sample distributes itself in both phases, it will migrate at an intermediate rate. This gas chromatography gives the total amount of vapour. Thus it is most widely used for quantitative analysis. GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal drugs [21, 22].

Hyphenated method such as GC-MS is commonly used for qualitative and quantitative analysis of phytochemicals [23, 27, 30]. GC-MS can be applied to solid, liquid and gaseous samples. First the samples are converted into gaseous state; and then analysis is carried out on the basis of mass to charge ratio. In recent investigation by the GC-MS analysis of *Cucurbita maxima* seed extract showed the presence of individual unsaturated fatty acids which were identified from Rf, peak area and by comparison of the data those reported in the literature [23].

Spectroscopy is used in the detection of phytochemicals present in medicinal plants [23–25].

Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known compounds and to elucidate the structure and chemical properties of molecules. The molecular weight of sample can be determined from MS Spectrum. Structural information can also be generated from certain types of mass spectrometers [23, 30]. This procedure is useful for the structural elucidation of organic compounds, for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterized compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously.

Ultraviolet and visible spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface [24]. Ultraviolet radiation is energetic enough to promote outer electrons to higher energy level and UV spectroscopy is usually applied to molecules or inorganic complexes in solution. This results from transition between the electronic energy levels. Measuring the absorbance at some wavelength by applying Beer-Lambert's law can determine the concentration of the analyte solution. It is useful to characterize the absorption, transmission and reflectivity of a variety of important materials such as pigments and other compounds from plants. This qualitative application requires recording at least a portion of the UV-Visible spectrum for characterization of the optical or electronic properties of materials. In recent experimental study, fluorescence and UV fingerprints were also developed for the methanol extracts of *Entada africana* leaf and stem. The UV absorption pattern for both plant parts identified that the leaf and stem bark extracts contain the same classes of constituents with extensive conjugated systems in their chemical structures. The most prominent peak with the strongest absorption was at 205–210 nm [24].

The fluorescence analysis of the drug powder is also used as a fingerprint for proper identification of crude drugs when other physical and chemical parameters of the crude drugs felt inadequate [17, 25, 31]. Fluorescence phenomenon exhibited by plant powder is primarily due to its chemical composition. The same material treated with various chemical reagents appears with different colours in different wavelengths of light. It has been reported that methanol, ethanol and acetone treated plant powders showed characteristic colour change when illuminated under UV light which is quite distinct from its colour observed under visible light. That marked changes in colours under UV light provide very distinct character which is specific to the crude drugs of this plant [26].

IR spectroscopy is used to determine the functional group present in the sample [27, 28]. Infrared absorption spectroscopy is the measurement of the wavelength and intensity of the absorption of mid-infrared light by a sample. Mid-infrared light is energetic enough to excite molecular vibrations to higher energy levels. The wavelength of many IR absorption bands are characteristics of specific types of chemical bonds, and IR spectroscopy finds its greatest utility for qualitative analysis of organic and organometallic molecules. Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds. The FTIR analysis confirmed the presence of N-H, O-H, C=C, C-H, C-O and CH_3 functional groups in recent phytochemical investigation of methanolic extract of *Curcuma caesia Roxb* [27]. In other investigation, the FT-IR spectroscopy studies of whole plant of *Aerva lanata* revealed the presence of functional groups, C=O, C-H, C=C, O-H, C-CHO, C-N and C-Cl in the plant [28].

Atomic absorption spectroscopy (AAS) is commonly used for elemental analysis of medicinal plants. In recent investigation using flame photometry and AAS for the estimation of various elemental concentration determined the level Fe, Cu, Mn, Zn, Ni, Mg, Mo present in medicinal plant by AAS flame/Graphite furnace with specific instrumental conditions as given by instruments' manufacturer [29].

According to recent report, finer analytical methods are now also available which can be incorporated to analyze the herbal drugs. Gel electrophoresis of isolated and purified DNA samples is done to identify the herbal drug as it cannot vary with factors like climate, etc. DNA fingerprinting can be made the choice of analysis for a perfect assessment of authentication of medicinal plant [32].

CONCLUSION

The result of this review showed that several analytical methods are employed and developed for standardization including chromatographic: TLC, GC, HPTLC and HPLC fingerprints, spectroscopic; UV, Fluorescence, MS, FT-IR, AAS and NMR are commonly applied techniques for different analytical purposes. The choice of analytical methods may depend on phytochemical, physicochemical nature of medicinal plant and scope of analysis. Development of analytical method is recommended for pesticide and aflatoxin analysis to further ensure purity, quality and for complete standardization of medicinal plants depending on history of exposure (Figure 4).

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