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Traditional Approaches towards Standardization of Herbal Medicines -A Review

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Abstract:
Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. Herbal medicines are gaining more and more attention all over the world, due to their long historical clinical practice and less side effects. This paper reviewed the traditional methods in the quality control of herbal medicines, including, the traditional chromatographic methods and comprehensive methods, such as fingerprint and multi-component quantification are emphasized; hyphenated techniques, like HPLC-MS, GC-MS. In a few word, the analysis and quality control of herbal medicines are moving towards an integrative and comprehensive direction, in order to better address the inherent holistic nature of herbal medicines.
Key words: *Herbal medicine, Quality control, Chromatography, Hyphenated technology*

Introduction:

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha, Unani, Homoeopathy and naturopathy. Traditional health care has been flourishing in this country for many centuries [1]. In India around 20,000 medicinal plant species have been recorded recently, [2] but more than 500 traditional communities use about 800 plant species for curing different diseases [3]. According to WHO, 70% of Indian population extensively use traditional and alternative medicines for health care [4]. The growing use of botanicals by the public is forcing moves to evaluate the health claims of these agents and to develop standards of quality and manufacture. Herbal products are widely perceived as being safe by patients because they are considering natural. Most medications before being offered to consumers undergo rigorous evidence-based clinical testing; this is not necessarily true for herbs. Consumers regularly use these products without the knowledge of their healthcare professionals [5].

Due to their long historical clinical use and reliable therapeutic efficacy, traditional Indian medicine attract and increase global attention, and many big pharmaceutical companies are using traditional Indian medicine as an excellent pool for discovering natural bioactive compounds. However, the characteristics of traditional Indian medicine are their systematism, multi-target and multi-channel due to their complex chemical constituents. If only few constituents are emphasized, the holistic nature will be neglected, which needs to be studied and scientifically understood. With the growing need for safer drugs, attention has been drawn to their quality, efficacy and standards of the traditional Indian medicine formulations [6, 7]. Each traditional system of medicines has their own method of standardization for assuring quality most in human linguistic terms. This method of evaluation has to be taken into consideration in standardization of herbal medicine [8].

Methods of identification, limitations and emerging techniques:

Most of the regulatory guidelines and pharmacopoeias suggest macroscopic and microscopic evaluation and chemical profiling of the botanical materials for quality control and standardization [9, 10, 11]. With respect to this, Department of AYUSH Govt. of India gave some parameters for Drug Development, Standardization & Quality of Ayurveda, Siddha and Unani drugs, which include five protocols as follows Protocol-I (Standardization of Single Plant Material), Protocol-II (SOP of Preparation of Extracts), Protocol-III (Standardization of Plant Extract), Protocol-IV (SOP of Finished Product) and protocol-V (Standardization of Formulations). These protocols based on

most common parameters such as morphological evaluation, microscopical evaluation, Physico-chemical evaluation, Particle size , Bulk density and Tap density in case of powder crude drugs or powder formulations, Assay for Constituents (Marker %, Major compounds like Alkaloids, flavonoids/saponin compounds), With respect to above parameters test for heavy/toxic metals (Lead, Cadmium, Mercury and Arsenic), Microbial contamination (total viable aerobic count, total Enterobacteriaceae and total fungal count), Test for specific pathogen (*E. coli, Salmonella spp., S. Aureus, Pseudomonas aeruginosa*), Pesticide residue (DDT, HCH, Endosufan, Alderin, Malathion and Parathion), test for aflatoxine (B1,B2,G1,G2) and Chelating agent (For bhasma, lepa, Aswarista etc.). Further stability assessment and self life, safety assessment, documentation of safety based on experience or toxicological studies, assessment of efficacy by ethnomedicinal informations and biological activity evaluation is essential. However, these parameters are judged subjectively and substitutes or adulterants may closely resemble the genuine material [12]. So chemical profiling is a essential parameter for standardization, which establishes a characteristic chemical pattern for a plant material, its fractions or extracts.

Qualitative and quantitative methods of Traditional medicines:

Chromatographic methods:

Chemical and chromatographic techniques may be used to aid in identification of an herbal material or extract. Chromatographic technique such as HPLC, TLC, GC and capillary electrophoresis and spectroscopic methods such as IR, NMR, and UV-may also be used for fingerprinting. DNA fingerprinting has been widely used in many species, e.g. DNA fingerprinting of Panax species and their adulterants [13]. Marker compounds may be used to help identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes and obtain stability profiles [14].

Central Council for Research in Ayurveda and Siddha gave list of phytoconstituents Shown in Table-1, which is used for analysis of crude drugs or herbal formulations containing these crude drugs.

TLC:

TLC is a simple, low-cost, versatile and specific method for the identification of herbal medicines. The unique feature of picture-like image of TLC supplies an intuitive visible profiling [13].

Table-1

List of phytoconstituents used for analysis of crude drugs or herbal formulations according to CCRAS.

Sl. No.	*Name of the compound*	*Source: Botanical name*	*Sanskrit/ Regional name*	*Part of the plant*	*Extract*
01	Atalantin	*Atalantia wightii* Tan. (Rutaceae)	-	Leaves and root bark	Chloroform and Ethylacetate
02	α-Amyrin	*Gmelina arborea* Linn.(Verbenaceae)	Gumbhari	Whole plant	Hexane
04	Amarogentin	*Swertia chirata* BuchHam. (Gentianaceae)	Kirata/Chir-ayita/Chireta	Aerial parts	Methanol
05	Aphanamixol	*Aphanamixis polystachya* (Wall.)Parker (Meliaceae)	Rohitaka/Tiktaraj	Stem bark	Methanol
06	Bergaptan	*Feronia limonia* Linn. (Rutaceae)	Kapitha/Kad Bel	Leaves	Hexane
08	Benzoic acid	*Shorea robusta* g.f.(Dipterocarpacea)	Shal	Stem bark	Ethylaceatae
09	Boehmeryl acetate	*Vanda roxburghii* R.br. (Orchidaceae)	Rasna	Whole plant	Hexane
10	Ceanothic acid	*Zizyphus jujuba* Lam.(Rhamnacea)	Badari	Stem bark	Ethylacetate
11	Clerodin	*Clerodendrum infortunatum* Linn. (Verbenaceae)	Bhant	Leaves	Hexane
12	Curcumin	*Curcuma longa* Linn. (Zingiberaceae)	Haridra/Haldi	Rhizome	Chloroform

Sl. No.	*Name of the compound*	*Source: Botanical name*	*Sanskrit/ Regional name*	*Part of the plant*	*Extract*
14	Embelin	*Embelia ribes* burm.f (Myrsinaceae)	Bidanga/Biranga	Seeds	Hexane
15	γ-Fagarine	*Glycosmis pentaphylla* Correa (Rutaceae)	Ashvashakota	Aerial parts	Methylene chloride
16	Geranyl umbelliferone	*Feronia limonia Linn .(Rutaceae)*	Kapitha/Kad Bel	Leaves	Hexane
17	Glycozoline	*Glycosmis pentaphylla Correa (Rutaceae)*	Ashvashakota	Aerial parts	Methylene chloride
18	Harmine	*Peganum harmala* Linn. (Rutaceae)	Isband	Seeds and roots	Chloroform
19	Isopimpinellin	*Feronia limonia* Linn. (Rutaceae)	Kapitha/Kad Bel	Leaves	Hexane
20	Koenimbine(MK-2)	*Murraya koenigii* Spreng. (Rutaceae)	Surabhinimba/Kurry patti	Seeds and leaves	Hexane
21	Lupeol(Crude)	*Diospyros peregrina* G. (Ebenaceae)	Tinduka/Gab	Stem bark	Petrol
24	Meranzin hydrate acetate	*Murraya exotica* Linn.. (Rutaceae)	Kamini	Leaves	Chloroform
25	Mangiferin	*Mangifera indica* Linn. (Anacardiaceae)	Amra/Aam	Unripe seed kernel	Alcohol
26	Murralongin	*Murraya exotica* Linn.. (Rutaceae)	Kamini	Leaves	Chloroform
27	Marsiline	*Marsilea minuta* Linn (Marsileacea)	Sunishanka/Susuni	Aerial parts	Hexane
28	Micromelumin	*Feronia limonia* Linn. (Rutacea)	Kapitha/Kad Bel	Leaves	Hexane
31	N-methyl-bi-cyclo atalaphylline	*Atalantia wightii* Tan. (Rutaceae)	--	Leaves	Chloroform
32	Oleanolic acid	*Swertia chirata* Buch.Ham.(Gentianaceae)	Kirata/Chirayita/Chireta	Aerial parts	Methanol
34	Osthol	*Feronia limonia* Linn.(Rutaceae)	Kapitha/Kad Bel	Leaves	Hexane
35	Premnazole	*Premna integrifolia* Linn.(Verbenaceae)	Ganakasika	Aerial parts	Solvent ether
36	Piperlongumin	*Piper longum* Linn. (Piperaceae) and *Piper nigrum* Linn. (Piperaceae)	Pippali/Pipul and Maricha	Fruits	Hexane
37	Piperine	*Piper longum* Linn. (Piperaceae) and *Piper nigrum* Linn. (Piperaceae)	Pippali/Pipul and Maricha	Fruits	Hexane
38	Phebalosin	*Murraya exotica* Linn.(Rutaceae)	Kamini	Leaves	Chloroform
39	Stearic acid	*Swertia chirata* Buch.Ham.(Gentianaceae)	Kirata/Chirayita/Chireta	Aerial parts	Hexane
40	Seselin	*Seseli indicum* W (Umbelliferae)	Vanayamini	Fruits	Hexane
41	Sitosterol	*Limonia crenulata* Roxb. (Rutacea)	Beli	Aerial parts	Petrol
42	Taraxasterol acetate	*Streblus asper* Lam. (Moraceae)	Shakhotaka	Aerial parts	Petrol
43	Tartaric acid	*Tamarindus indica* Linn. (Leguminosae)	Tintrini/Tamarind/ Tentul	Fruits	Alcohol
44	Umbelliferone	*Atalantia wightii* Tam. (Rutaceae)	--	Leaves	Chloroform
45	Xanthotoxin	*Limonia crenulata* Roxb. (Rutaceae) and *Feronia limonia* Linn. (Rutaceae)	Beli And Kapitha/Kad Bel	Leaves	Petrol
46	1,5,8-trihydroxy-3-methoxy xanthone (1^{st} lot)	*Swertia chirata* Buch.Ham.(Gentianaceae)	Kirata/Chirayita/Chireta	Aerial parts	Hexane
47	1,5,8-trihydroxy-3-methoxy xanthone	*Swertia chirata* Buch.Ham.(Gentianaceae)	Kirata/Chirayita/Chireta	Aerial parts	Hexane
48	1,3,7-trimethoxy-8-hydroxy xanthone	*Swertia chirata* Buch.Ham.(Gentianaceae)	Kirata/Chirayita/Chireta	Aerial parts	Hexane
49	1,8-$(OH)_2$-3,5-$(OMe)_2$ xanthone	*Swertia chirata* Buch.Ham.(Gentianaceae)	Kirata/Chirayita/Chireta	Aerial parts	Hexane
50	1,3,7,8-tetrahydroxy xanthone	*Swertia chirata* Buch.Ham.(Gentianaceae)	Kirata/Chirayita/Chireta	Aerial parts	Hexane
51	1,8-$(OH)_2$-3,7-$(OMe)_2$ xanthone	*Mangifer indica* Linn. (Anacardiaceae)	Amra/Am	Aerial parts	Hexane

Nowadays HPTLC is a routine analytical technique.It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC [15, 16]. It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation [17, 18, 19]. With this technique, authentication of various species of plant possible, as well as the evaluation of stability and consistency of their preparations from different manufactures.

Various workers have developed HPTLC method for phytoconstituents in crude drugs or herbal formulations such as bergenin, catechine and gallic acid in *Bergenia cilliata* and *Bergenia lingulata* [20], quercetin-3-O-β-D-rhamnoside in *Euphorbia hirta* [21], Withaferine-A in Withania somnifera [22], caffine in herbal products and power drinks [23], 14-deoxy-11,12-didehydroandrographolide, andrographolide, neoandrographolide and andrographoside in *Andrographis paninculata* [24], capsacine and piperine in Milangi thailam [25], genistein and daidzein in *Glycine max* [26], bergenin in *Caesalpinia digyna* [27], artemisinin in *Artemisia annua* L.[28], betulin and betulinic acid in herbal formulation Virala [29], leuteolin in *Thymus vulgaris* [30], aescin in herbal medicinal products containing Aesculus and vitis extract [31], curcumin in herbal formulations [32], picroside-I and picroside-II in Picrorhiza kurroa [33], theophyllene and etofylline in pharmaceutical dosage form [34].

HPLC and hyphenated techniques:

High-performance liquid chromatography (HPLC) has been employed to analyze several components in a medicinal preparation composed of several crude drugs [35,36,37]. Among the analytical methods for standardization of Indian herbal medicines HPLC is the most popular one, due to its versatility, precision and relatively lowcost. [38,39,40]. Which represents a progress in comparison of the one or two marker quantitative approach. One of the main advantages of HPLC is that many detectors can be coupled with it, such as UV, DAD, ELSD, FLD, RID, MS, and NMR, etc., which supplies much more possibilities for detecting different constituent types. In recent years, coulometric electrode array detection (HPLC-CEAD) and charged aerosol detection (CAD) [41, 42] have been also introduced to the analysis of herbal formulations. Most frequently, the method is used on reversed phase (RP) C18 columns, a binary solvent system containing acidified water, a polar organic solvent (acetonitrile or methanol), and UV–vis diode array detection (DAD). HPLC method with various detectors has been developed for qualitative and quantitative analysis of various phytoconstituents such as chiconic acid in *Posidonia oceanica* (HPLC-UV detector) [43], simultaneous determination of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin in kampo medicines (HPLC-photodiode array detector) [44], phenolic compounds in *Achillea millefolium* L. (reverse phase HPLC-dual λ absorbance detector such as UV/Vis and Photodiode arrey) [45], Chlorogenic acid in Tobacco residuals (HPLC system with UV detector and C18 reversed phase column)[46] and phenolics in chyavanpras (RP column with HPLC UV–vis diode array detection) [47].

GC and hyphenated techniques:

GC and GC-MS are unanimously accepted methods for the analysis of volatile constituents of herbal medicines, due to their sensitivity, stability and high efficiency. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the complex constituents [48, 49]. Nowadays, the analysts turn to gas chromatography as a powerful separation method and combined it with mass spectrometry to aid identification. The GC–MS technique, along with improved data handling tools-will immediately be relevant to the essential oil area [50]. A number of detectors are used in gas chromatography. The most common are the flame ionization

detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before an FID (destructive), thus providing complementary detection of the same analytes.

In recent years so many workers developed GC or GC hyphenated with MS for the analysis of phytoconstituents such as thymol, eucalyptol, menthol, camphor from honey and bees wax (Gas chromatography with flame ionisation detection) [51], estragole, safrole and eugenol methyl ether in food products (Gas- chromatograph directly interfaced with GCQ plus mass spectrometer) [52], eugenol (GC with flame ionisation detector)[53] and sugars (Gas chromatograph interfaced with mass selective detector)[54].

Comprehensive methods:

Fingerprint:

The fingerprint has potential to determine authenticity and reliability of chemical constituents of herbal drug and formulations [55]. It addresses the systematic and comprehensive nature of herbal medicines, so it has been internationally accepted as one of the efficient methods to control the quality of herbal medicines [56]. Several reviews on fingerprint have been published [57-60], so, here, new progress of this technique has been concerned. Chromatographic and spectroscopic technologies are still two main methods for establishing the fingerprint, including TLC, HPLC, GC, CE, IR, NIR, NMR, as well as DNA fingerprinting. Nowadays, more and more hyphenated technologies are used to obtain much more information, such as GC-MS, HPLC-MS, HPLC-DAD-MS, and LC-NMR. Especially, the hyphenation of MS with HPLC or GC has been a very useful means to the chemical constituents' analysis, quality control and metabolite studies, etc. [58,61]. Sometimes, a single fingerprint is inadequate for the effective analysis of complex herbal medicine, so multiple chromatographic fingerprints with different test conditions in the same or different separation principles have been proposed.

Conclusion:

Herbal drug standardization is massively wide and deep. Quality control of herbal medicines has not only to establish reasonable analytical methods for analyzing the active constituents in herbal medicines, but many other factors should be concerned, such as pesticides residue, aflatoxine content, the heavy metals contamination, good agricultural practice (GAP), good manufacturing practice (GMP), etc. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. Among the existing analytical methods, chromatographic methods are still the mainstream, but more and more spectroscopic methods have also been developed, due to their integrative evaluation characteristics. Now a day's importance is given on chemical constituents and negation of human cognitive process. Is this justifying the traditional medicine? There is need for development of techniques which includes both traditional methods of evaluation and modern methods of evaluation. This will improve the quality of the drug and also motivates the practitioners to get more involved in the standardization process.

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