Pharmacogn. Res., 2024; 16(4):677-687.
https://www.phcogres.com

Review Article

HPLC Based Metabolomic Fingerprint Profiling as A Comprehensive Quality Assessment Tool, Why Not for Standardization of Ayurvedic Herbs and Formulations?

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ABSTRACT

A global resurgence and rampant increase in the usage of herbal medicines has led to malpractices in the absence of adequate regulatory control to meet the growing demand. To ensure quality and hence efficacy, of these drugs is the prime concern. The reason for poor regulatory control over herbal drugs is lack of adequate and accepted methods for evaluating them. In spite of technological advancement in analytical methods and instruments, standardization of herbal drugs is still an elusive goal. High Performance Liquid chromatography based metabolomic fingerprint profiling approach for different herbs and herbal formulations can be answer to this. HPLC based this approach is robust, precise, quantitative, easy to use, thus safeguarding that herbal medicine can be authenticated for their quality evaluation. In this review an introduction is given that why the quality evaluation of herbal medicine is important by means of HPLC based metabolomics. This approach includes extraction, chromatographic analysis and chemometric analysis to the produced chromatographic data. Various studies based on this approach are discussed that can be the torch light and helpful to keep the Legacy of intangible ayurvedic culture intact, that why and how this approach should be adopted.

Keywords: Ayurveda, Quality, Metabolomics, Fingerprint, HPLC, Chemometric.

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Received: 08-04-2024;
Revised: 04-06-2024;
Accepted: 14-06-2024.

INTRODUCTION

Healthy life and well-being at every age, United Nation's second sustainable development goal is one of the major goals in today's modern world. To ensure this apart from available medicinal system, plants have been used as a source of medicine since the existence of mankind. Ayurvedic system of medicine enlightens the use of plants as a treatment for various human ailments. Mostly, the phytomedicines are consumed in the form of fresh or dried part/s of plant, powder, tablets and capsules for benefit of one's health. The popularity of herbal medicine is consistently increasing on account of their ease of availability, economic and harmless nature. The traditional herbal medicines and their preparations have been extensively used for thousands of years in many countries like India, China, Korea, Japan, etc. The preparation of herbal medicine usually involves the use of one or more parts of herbs boiled in predetermined quantities of water to prepare its decoction. This is the motive why quality control of herbal drugs is more challenging than that of the synthetic drugs. As pointed in "General guideline for Methodologies on Research and Evaluation of Traditional Medicines (World Health Organization, 2000)", despite the worldwide accepted use of herbs as medicines; there exists a lacuna when it comes to quality control of these multi-component formulations. The lacuna could be due to not only health care policy but also due to the lack of established research methodology.[1-4] Herbs can be easily differentiated morphologically when they are in intact form, but the crude drugs sold commercially, are generally dry and fragmented and therefore, difficult to identify. When they are traded in the powdered form, it becomes even more difficult to identify them because market samples are invariably adulterated and according to one report, more than 60% phytochemical examination carried out on market samples of Indian herbs are based on incorrect/adulterated samples.[5] Raw material which is used for manufacturing herbal formulations, should be of good quality to be effective. Proper identification of the raw materials is of prime importance to ensure efficacy of the finished product.

DOI: 10.5530/pres.16.4.79

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

Apart from this, effectiveness of an herbal drug also depends on place of collection, season, age of plant when collection is done and on proper storage of collected drugs. Several Ayurvedic texts such as Charaka Samhita, Sushrut Samhita, Ashtang Hridaya and Sharangdhar Samhita have emphasized the importance of proper collection and storage of crude drugs. Chemistry of plant drugs may differ with various factors like growing stage, harvest time, location, storage condition, processing and manufacturing procedures, but they can yield robust information to address adulteration issues for quality assurance in herbal industry.[6] India is blessed with the ancient heritage of Ayurveda, which is a science of holistic healing. The vast biodiversity of plants and the ancient science of Ayurveda give our country, an edge over others, in becoming global leader in holistic healing through herbal medicines. A global resurgence in the use of herbal medicines in the past couple of decades has set the stage for India. However, rampant increase in the production of herbal drugs to meet the global demand has led to malpractices in the absence of adequate regulatory control to ensure quality and hence efficacy, of these drugs. The reason for poor regulatory control over herbal drugs is lack of adequate and accepted methods for evaluating them. In spite of technological advancement in analytical methods and instruments, standardization of herbal drugs is still an elusive goal, because of variations in the chemical constituents of herbs. These variations may be geographical, seasonal, diurnal or genetic (Chemotypes). In case of polyherbal formulations, further variations are introduced due to adulteration, substitution or omission of an ingredient and also due to batch-to-batch variation in process parameters.

The approach used so far for quality control of herbal drugs includes quantification of one or more bioactive constituents in it. However, this approach does not give a comprehensive picture of the quality of the herb, since, in most cases, the activity of herbal drugs is not due to one or two compounds but it is concerted effect of many compounds present in it. Hence, a more scientific and logical approach would be to consider the entire metabolome of the herb, in order to assess its quality. Metabolism within the cells of an organism is a product of its genome and habitat and all metabolites synthesized by the organism form its *metabolome*.[7] Since the metabolome differs for each species and even for organisms and even for organisms in the same species, it can be exploited to make a distinction between subjects. Studying the profiles of all or a significant number of cell metabolites is known as *metabolomics*.[8] Alternatively, such studies are also commonly called *metabonomics*.[9-11]

Fecund increase in usage of herbs and herbal formulations ultimately deal with health of human beings. This will directly impact United Nation's sustainable development goal-3, Ensure healthy lives and promote well-being for all at all ages. Increasing demand of herbs and herbal formulations will also instigate to malpractice to get maximum benefits in terms of money by substitution, omission and addition of exhaustive related herbs. This will indirectly affect United Nation's sustainable development goals-14, 15 and 13; Life on Land, Life below water and climate change respectively. So, metabolomics approach used to access the quality of the Herbs and herbal formulations based on various key issues can be the complementary option for above discussed United Nation's sustainable development goals. Here, various studies based on HPLC metabolomic fingerprint profiling approach are discussed that can be the torch light and helpful to keep the Legacy of intangible ayurvedic culture intact, that why and how this approach should be adopted.

Experimental

Metabolomics is an inclusive analysis of whole metabolites of a biotic system. Despite the critical appeals of some experts, most of the scientific community in the field seems to consider "metabolomics" simply as study of metabolome. For metabolomics analysis, several analytical techniques have been used, such as Gas Chromatography (GC), Liquid Chromatography (LC), Fourier Transform Infrared (FTIR) spectroscopy, Mass spectroscopy and NMR spectroscopy. High Performance Liquid Chromatography (HPLC) is one of the leading methods used for separation of compounds present in a mixture. This method works based on the interaction between a liquid mobile phase and a solid or fixed gel stationary phase.

It is very efficient and yields excellent separations in a very short time. Diffusion coefficient is much less than other chromatographic techniques. The compressibility of the mobile phase under pressure is negligibly small. Analyte detection up to picograms is possible. Sampling is very easy and not problematic with HPLC technique. Thermolabile compounds can be analyzed at ambient temperatures. Aqueous solvent can be used, so major sample pretreatment is not required. Automatic sample injection devices allow many samples to be analysed without operator intervention.[12-14]

In metabolic fingereprinting, Extract is being prepared for herbal medicines. This extract is then treated to appropriate chromatographic and spectroscopic method. This generated data exhibits whole spectra or chromatograms that consist of vast amount of peak and intensity values. To analyze the irresistible expanse of data, computational pattern recognition methods are utilized. Pattern recognition practices apply precise and statistical methods to summarize the data and to extract meaningful data from the bulk data. Analyzing chemical data with mathematical or statistical methods is an arena of science known as chemo metrics,[15] often also referred to as multivariate statistical analysis,[16] although the latter term is more general and does not confine the data to be chemical data. Furthermore, to the fields of metabolomics and metabonomic, chemo metrics are commonly applied to quality control purposes. When cheap inauthentic products are labelled as expensive brands, the fraud may be

distinguished by chemo metric analysis. Unsupervised pattern recognition techniques are often used to see similarities and dissimilarities between samples. It is interesting to see if some samples are clearly different from the rest. Such result would raise questions like what these samples have in mutual and what makes them special and launch investigations that never would have been carried out without knowing the hidden pattern in the data.[17] Schematic diagram is represented to engulf the HPLC based metabolomic Fingerprint in Figure 1.

DISCUSSION

HPLC based metabolomic Fingerprint Profiling for Quality assessment

Quality for the herbs and herbal formulation evaluated in various studies that can be the torchlight to adopt this approach for the glorious legacy of ayurvedic herbs and polyherbal formulations to justify their authenticity. Few case studies are discussed here where this approach is well depicted.

Ting hun lee *et al.* has developed the quality estimation technique for the Edible Birds Nest (EBN) used mainly as functional food native to Malaysia. His intention was to discriminate EBN of West Malaysia and East Malaysia on the basis of amino acid composition. Using HPLC and multivariate analysis tools such as PCA, OPLS DA and HCA, he was able to successfully quantify 18 amino acids from EBN and quantified 4 amino acids as the biomarkers for discrimination between West Malaysia and East Malaysia.[18] Du jing *et al.* analysed a very big domain of interest which is plant medicines and it is gaining more and more attention in all over the world. Many modern analytical techniques along with the chemometrices and multivariate analysis are useful in quantifying, assessing and evaluating the authenticity, safety and efficacy of the medicinal plants.[19] Lingfeng wu *et al.* has developed method for quantifying the phenolic compounds present in the mango leaves. This study checked the correlation between the antioxidant activity and in silico α glucosidase inhibitor ability of the different mango leaves varieties collected from different places. Neomangiferin, mangiferin, kaempferol-3-O-rutinoside and quercetin were identified as a potential biomarker. Pearson correlation coefficient analysis confirms that these compounds directly contribute to the antioxidant activity and α glucosidase inhibitor activity.[20] Abdelkrim rebiai *et al.* succeeded in developing a method for quantifying bee pollen quality as it varies with the geographical location change. This study aimed to apply brisk, easy and precise analytical methods like HPLC and ATR-FTIR along with the chemometrics methods to develop a model to discriminate between different pollen varieties. In all 33 samples were analysed using HCA, PCA and PLS for assess similarities and differences between the samples also the samples were discriminated in 4 groups on the basis of their total polyphenolic contents.[21] LC Salanta *et al.* used RP HPLC for the characterization of the hop varieties obtained from the *Romania*. The aim of this study was to study the evolution of bitter acids from the three varieties of hop growing in *Romania* during the development of hop cones and palletisation process in order to provide information on bitter acids profiles. Chemometrics methods were applied for assessing the correlations existing between the genotype, phenotype and chemotype on the basis of bitter acids content.[22] ShaoJing Liu *et al.* has developed the chemical fingerprinting and analysis of

Figure 1: Key steps of HPLC based metabolomic fingerprint profiling for quality assessment of herbal medicines.

Paeonia ostii leaves. 35 leaves collected from the four provinces of the China were collected and divided into 4 geographical groups by multivariate analysis. From those 56 chemical components were identified by UPLC-ESI Q/TOF-MS/MS, seven characteristic components in the 35 samples were quantified. The HPLC-DAD fingerprinting were combined with the SA, PCA, HCA and OPLS- DA were useful for evaluating the differences between chemical composition of the 35 samples.[23] Jing Hu *et al.* characterized raw and stir frying processed *Drynariae rhizoma*. According to the Traditional Chinese medicine the raw (RDR) and stir-frying Processed (PDR) *Drynariae rhizoma* have different clinical applications. The aim of this study was to establish the HPLC fingerprints coupled with chemometric methods to evaluate the differences between RDR and PDR. The results of PCA, HCA and PLS DA indicated a clear separation between the two varieties. This study identified seven chemical markers contributing a greater extent for the separation of this varieties.[24] Ben li *et al.* utilized the HPLC DAD fingerprinting method for the analysis of the epimedii folium species. In this study nine sets of the plant species from the different locations were collected and analysed using HPLC-DAD, the objective of this study was to investigate the effect of the geographical origin and *Epimedium* species on quality of *Epimedium folium*, the plant species were used to establish HPLC-DAD fingerprints and 91 peaks selected out of which major compounds were analysed by PCA. The study showed that the major bioactive compound is responsible for the influence of geographical origin and quality of the species.[25] M. Rahimmalek *et al.* had studies on the *Lamacieae* species which is a major species for the medicinal plants and are valued for their antioxidant properties and multiple pharmaceuticals uses in folk medicines. The present study involved using the seven Iranian endemic species for the comparative studies of their antioxidant properties. Three species were selected out of seven and used for detection of the antiglycation properties. Out of all the species *S. hortensis* possessed the highest antioxidant and anti glycative activity among all studied species. Rosmarinic acid was identified as a predominant phenolic compound in all species.[26] Qian Liu *et al.* identified that there was no proper method for the simultaneous determination of the 17 polyphenols in six Chinese raw propolis samples using chemometrics assisted HPLC-DAD method was developed and validated. The calibration method was based on the ATLD algorithm, f-test and t-test were carried out on the data obtained from MRM mode and showed 95% confidence level and indicated a suitable tool for the rapid analysis of the Chinese raw propolis.[27] Dimitrina Zh. Zheleva-Dimitrova *et al.* investigated *Arnicae flos* flowers obtained from various places like Bulgaria, Poland, Germany, Finland and Pharmacy store for analysis of phenolic derivatives and sesquiterpene lactones. The aim of this study was to extract and analyse phenolic acids and flavonoids simultaneous determination of this secondary metabolites. PCA and HC clusters were used for the distinguishing of the flavanols in the *Arnicae montana* flowers.[28] Radamés Alvarez-Zapata *et al.* used chemometrics in enhanced HPLC-UV for the phytochemical detection of bioactive metabolites in phytochemically unknown plants. They have used Colubrina gregii as a model and combined data obtained from the leishmanicidal bioassay using PCA and OPLS-DA methods. This study incorporated that chemometrics analysis with bioassay are the best tool for the detection and isolation of bioactive phytochemically unknown medicinal plants.[29] Xiao-Hua Zhang *et al.* researched on the quantitative analysis of the bioactive constituents i.e. flavonoids in the Chinese propolis, they investigated the application of the chemometrics coupled with HPLC DAD technique for the rapid and sensitive quantitative analysis of seven flavonoids in raw and purified propolis. For validation of the method ATLD algorithm was used. Total content of the flavonoids in the raw propolis was less as compare d to the purified propolis.[30] Josep Pages-Rebull *et al.* explored the novel method for the authentication of the aromatic herbs and spices by incorporating chemometrices method. The aim of this study was to develop a HPLC-UV coupled chemometrices method for the spice's identification and authentication. Phenolic profiles based in six characterisitcs biomarkers were considered in this study and eight types of spices or aromatic herbs were discriminated.[31] Naser Gholizadeh-Moghadam *et al.* classified the barberry genotypes on the basis of their biochemical constituents. Due to their promising edible properties and growing interests as the anti-oxidants in food and pharmaceutical products was the basis of this study. 18 fruit samples were utilized in this study for the genotype classification of the barberry using the multivariate analysis. Using this study many fruit extract varieties were classified on the basis of total phenolics and total flavonoids.[32] Mohler Smith *et al.* used various cheese varieties by using multivariate analysis of HPLC profiles. The aim of this study was to analyze water extracts of cheddar, edam, gouda, swiss and parmesan cheese varieties using the RP- HPLC method. PCA and DA analyses were applied to the 55 peaks of the cheese varieties. DA analysis was used to successfully discriminate cheese varieties at the success rate of 90 %.[33] Soumeia Zeghoud *et al.* developed classification of plant medicine species native to Algerian region using various analytical techniques like UV and HPLC combined with the chemomterices analysis. The aim of this study was to develop an easy and accurate analytical approach for classifying many plants using PCA, HCA, PLS-DA and PLS tools. Based on this study and obtained findings the closely related species may be identified and differentiated using above mentioned methods.[34] Mohammad saadatian *et al.* found out that pomegranate fruits are highly diverse and divided into geographical groupings on the basis of their characteristics. This aimed to assess the eight varieties of the pomegranate cultivars using multivariate statistical analysis. Shaqlawa, Halabja Sour and Faqyan pomegranate cultivars were found to have highest antioxidant activities. The results of the multivariate analysis showed that pomegranate cultivars were divided into 4 major

groups.[35] Song JH *et al.* discovered that there was no validated method for the discrimination of the *Coptis chinensis* Rhizome and fibrous roots. So to eradicate these problem they developed a method in combination with the HPLC and chemometrics analysis using SA, HCA, PCA, OPLS- DA.[36] W.W. Dong *et al.* identified the method for the discrimination of the *Astragali radix* from its adulterants on the basis of their active biochemical constituent. They developed a method on the basis of the HPLC coupling with the chemometrics tools like HCA and PCA for developing the felicitous method for the adulteration determination in the species.[37] P. de la Mata-Espinosa *et al.* delve into the problem of discriminating the olive and non-olive oils. Their work represents an efficient method for the determination of the different vegetable and olive oil using chemometrics tools. Typical chromatograms of the triacylglycerides were analysed using PCA, PLS-DA and MCR in combination with suitable pre-processing. The findings showed altruistic results for the separation of the olive and vegetable oils.[38] Piotr Marek Kuś *et al.* learned that discrimination of the unifloral honey is indeed a provocation. He harnessed total 62 honey samples of six floral origins by PTR-MS and HPLC-DAD. Obtained data was evaluated using PCA and k-nearest neighbours' technique to evaluate the consistent fingerprint between various species of honeys. The classification data obtained from HPLC was more promising as compared to that of the PTR-MS but it can be used as an efficient method for the fingerprinting of the other species.[39] Yanli Zhao *et al.* evaluated the quality of the wild paris species of different regions to investigate whether the quality is dependent on the region. NIR spectroscopy and HPLC was used for the bioactive constituent separation. The obtained data was studied, integrated and analyzed using PLS-DA method. Spectroscopic and chromatographic methods were able to distinguish the various regional varieties.[40] Freddy a. Bernal *et al.* experimented the piper plant extract analysis coupled with the chemometrics. 23 ethanolic extracts of the piper plant species were collected and analysed using HPLC -UV-DAD. The data obtained was assessed by direct two-way method using PCA and HCA and after HCA data minimization three-way method using PARAFAC. The collected samples supported the previous finding of the piper plant species and also suggested a specific phylogeny relationship between among the piper samples.[41] Nikunj Patel *et al.* ascertained that there was no method for the geographical classification of the *Adhatoda vasica* samples. The aim of their study was to geographically classify the adhatoda vasica species collected from the different regions of the India, HPLC fingerprinting analysis coupled with multivariate analytical tools like PCA was performed. PCA model successfully classified all the collected 16 samples based on their geographical regionality.[42] Stelios Chatzimichail *et al.* discovered that there was no proper method for the detection of the Polycyclic Aromatic Hydrocarbons (PAH) in any samples though they were carcinogenic. They developed a hand portable HPLC method for the identification of the PAHs. The MCR- AR technique was used to detect the amount of the PAHs also the species were classified using spectral fingerprinting. Using this method an automated, unsupervised detection classification and characterisation of the species was possible.[43] M. Nurul islam *et al.* investigated the estrogenic activity of the Epimedium koreanum extract. 31 samples were evaluated by a luciferase reporter gene assay and samples were classified in 3 groups based on their bioactivity. Fingerprint analysis was performed on the data obtained from the HPLC from which 44 peaks were selected and canonical discriminant analysis was performed, 4 major extract compound showed good correlation with estrogenic activities.[44] Nikunj patel *et al.* evaluated the quality of the kutki samples for the discrimination of the species. To do so RP-HPLC fingerprinting method coupled with PDA was established. The obtained findings were evaluated along with multivariate analysis and was capable for discriminating the collected 11 samples. Also, the chemometrics techniques like PCA and HCA were applied to identify different regional samples of the kutki samples.[45]

Shihan Wang, Yang Xu, *et al.* evolved a comprehensive method for the quality evaluation and quality assessment of the *Oviductus ranae*. The fingerprinting of the plant speices were done using HPLC. The HPLC profiles of the 14 batches were performed and showed 33 well reserved common peaks in the chromatogram. The HPLC data was analyzed using HCA and showed that sample classified into one group showed the relation with habitats.[46] Agnieszka Viapiana *et al.* developed a study relating to the quality consistency evaluation of the *Sambucus nigra* plant species. The HPLC profiles of 13 batches showed more than 60 peaks from which 14 well resolved peaks were selected. The HCA and PCA analysis showed similar chemical composition reflected in 14 common peaks showing similarity values.[47] Puigventos *et al.* developed a method for the adulteration identification of the cranberry-based products. In order to identify the adulteration of the cranberry-based products with grapes chemometrics method like PCA and PLSR was used. The adulteration was quantified using HPLC- UV polyphenolic fingerprints. The proposed method resulted in the excellent approach for the detection of the adulteration.[48]

Ting Wang *et al.* analysed the problem related to the quality identification of the *Corni fructus*. The method was developed using HPLC DVD along with chemometrics tools comparing the Q marker content and colour of the corni fructus fruit. The correlation was studied by using MLR, BP- ANNs and SVM and they established a Q marker colouring card with best fitting degree according to the predication model.[49] Abdul Rohman *et al.* developed a method for the calibration of the andrographolide in the *Andrographis paniculata* extract. The andrographolide was quantitatively analyzed by reference method HPLC using multivariate analysis, the obtained data was studied using RMSEC, RMSEP and PLSR method; proved to be the alternative

Table 1: HPLC based metabolomic fingerprint profiling for quality evaluation of various herbal plant medicines.

Sl. No.	Compound/ extract	Detector	Method	Type of analysis	References
1	Edible birds nest	Fluorescence detector	HCA, PCA, OPLS-DA	Amino acid determination.	18
2	Medicinal plants	DAD	PCA, PLS, HCA, SA	Quality evaluation of medicinal plants.	19
3	Mango leaves	Electrospray Ionization (ESI)	Pearson correlation coefficient analysis, DA	Phenolic compounds analysis and their antioxidant activity and in silico α-glucoidase inhibitory ability correlation.	20
4	Honeybees	UV detector	PCA, PLS, HCA	Bee pollen quality analysis.	21
5	Hop varieties of *Romania*	UV detector	CA, PCA	Hop varieties characterization based on bitter acid content.	22
6	*Paeonia suffruticosa* Andr. (Tree peony)	DAD	SA, PCA, HCA, OPLS DA	*Paeonia ostii* leaves chemical fingerprinting.	23
7	Drynariae Rhizoma	PDA	HCA, PCA, PLS DA	Characterization of Raw and Stir-Frying Processed.	24
8	Epimedii folium	DAD	PCA	Investigate the influence of geographical origin.	25
9	*Lamiaceae* species	UV detector	HCA, CA	Variations in polyphenolic compounds and antioxidant and antiglycative activities of species native to iran.	26
10	Raw propolis	DAD	ATLD	Simultaneous determination of polyphenols without interference.	27
11	Arnicae flos	UV detector	HCA, PCA	Investigate phenolic derivatives and Sesquiterpene Lactones (STLs) content of different areas varieties.	28
12	Colubrina gregii	UV detector	PCA, O-PLS	Bioactive metabolite detection.	29
13	Chinese propolis	DAD	ATLD	Simultaneous estimation of flavonoids in plant.	30
14	Cinnamon, Oregano, thyme, Sesame, Bay leaf, Clove, Cumin and Vanilla.	UV detector	SIMCA, PLS, PCA	Characterization, identification and authentication Aromatic herbs and spices.	31
15	Barberry fruits	UV detector	PCA, HCA	Evaluation of biochemical constituents and classification of genotypes.	32
16	Cheddar, Edam, Gouda, Swiss and Parmesan cheeses	VWD (Variable Wavelength Detectors)	PCA, DA	Cheese varieties classification.	33
17	Medicinal plants	UV detector	PCA, PLS, HCA, PLS DA	Various medicinal plant species classification collected from the Algerian region.	34

Sl. No.	Compound/ extract	Detector	Method	Type of analysis	References
18	Eight varieties of pomegranate fruit extracts	DAD	PCA, HCA	Classification of pomegranate fruits by antioxidant activity.	35
19	Coptis chinensis	UV detector	SA, HCA, PCA, OPLS DA	Discrimination of rhizome and fibrous root.	36
20	Astragali radix	DAD	PCA, HCA	Species Discrimination from its adulterants.	37
21	Varieties of vegetable oils	Charged Aerosol Detector (CAD)	PCA, PLS-DA, MCR	Discrimination of olive and non-olive oils.	38
22	Rapeseed, lime, heather, cornflower, buckwheat and black locust honeys	DAD	PCA, k-nearest neighbour classification	Discrimination of unifloral regions honeys.	39
23	Wild paris species	DAD	PLS DA	Discrimination of different varities of species of different geographical origin.	40
24	*Piper* plant species	UV, DAD	HCA, PCA, PARAFAC	Fingerprint analysis of unfractioned species.	41
25	Adhatoda vasica	Dual lambda absorbance detector	PCA	Geographical classification of the species.	42
26	Polycyclic aromatic hydrocarbon mixtures	UV detector	MCR-AR	Hand portable system enables broadband spectral detection of PAHs.	43
27	*Epimedium koreanum* extract	UV detector	PRA	Predict the Estrogenic Activity of extract.	44
28	Picrorhiza kurroa	PDA	HCA, PCA	Fingerprint analysis for quality determination.	45
29	Oviductus ranae	DAD	HCA	Quality evaluation.	46
30	*Sambucus nigra* L. berries	UV detector	HCA, PCA	Quality consistency evaluation.	47
31	Cranberry extracts and red cranberry extract	UV detector	PLSR, PCA	Fingerprinting and authentication of fruits.	48
32	*Corni fructus* fruit	DVD	MLR, BP-ANNs and SVM	Q marker content analysis.	49
33	Andrographis paniculata	UV detector	PLSR	Calibration of andrographolide.	50
34	77 varieties of honey from 18 regions	ECD	PCA- DA	Identification of monofloral honeys.	51
35	Agarwood samples	UV	PLS- DA, fisher linear recognition.	Identification of wild and cultivated species.	52
36	*Hedyotis diffusa* Willd.	DAD	SA, HCA, PCA, OPLS-DA	Differentiation between wild and cultivated species.	53
37	Citrus species	RID	HCA	Metabolic fingerprinting of citrus cultivars and related species.	54
38	MOOCA	DAD, UV	PARAFAC	Open-source python software application for quality assessment.	55

Sl. No.	Compound/ extract	Detector	Method	Type of analysis	References
39	*Poria cocos* and *Polyporus umbellatus.*	DAD	HCA, PCA, LDA	Monosaccharide composition of polysaccharide present in it.	56
40	Sucrose regioisomers	CAD	PLS	Sucrose monoester regioisomers detection.	57
41	Fermented food	DAD	U-PLS/RBL, PARAFAC	Quantitative evaluation of short chain organic acids.	58
42	Pistachio nuts	UV detector	PCA, AHC	Determination of the bioactive compounds for quality authentication.	59
43	Durum wheat	UV detector	PCA	Quality study of the species.	60
44	Saffron	UV detector	SA, PCA, HCA, OPLS-DA	Relationship between HPLC fingerprints and antioxidant activity of species.	61
45	Cannabis	PDA	PCA	Physical analysis and chemical profiling of illicit herbal samples.	62
46	Trifoliate orange	DAD	HCA, PCA, PLS-DA	Phytochemical analysis during fermentation.	63
47	Cannabis	PDA	CA, SIMCA, PCA	Drug profiling of the species.	64
48	*Potentilla fruticosa* L.	DAD	SA, HCA, PCA, DA	Quality evaluation and chromatographic fingerprinting.	65
49	*Dendrobium Nobile* Lindl.	UV detector	CA, PCA, OPLS-DA	Fingerprinting of the flower.	66
50	Curcurmae rhizhoma	UV detector	DT, LDA, SVM, KNN, PLS, BP-ANN	Rapid discrimination and quantitation of three species of botanical origins.	67

method for the quantitative analysis of the Andrographolide.[50] Jing Zhao *et al.* analysed potential floral markers of the monofloral honeys using HPLC-ECD method. Fingerprinting analysis of the honey samples was established and classification was performed using phenolic acids and common chromatography peaks. 41 test specimens were analysed using chemometrics PCA and DA model and it was successfully able to classify the honey samples.[51] Shang Lili *et al.* developed a method for the identification method of wild and cultivated agarwood samples. The analysis was done using HPLC fingerprinting analysis coupled with multivariate data analysis using PLS-DA model. Nine chromatographic peaks were selected and they showed promising separation between the cultivated and wild agarwood samples. Based on discriminant analysis classification accuracy was reached 100 %.[52] Xin Wang *et al.* identified the problem to separate wild and cultivate *Hedyotis diffusa* willd (HDW). HPLC fingerprinting method analysis was performed and nine major components were successfully identified as a discriminant tool between wild and cultivated *Hedyotis diffusa* species. The species were also characterized using multivariate analysis tools like SA, HCA, PCA and OPLS-DA to characterize the differences in chemical compositions between wild and cultivated plant species. This study provided suitable method for germplasm discrimination and quality evaluation of HDW.[53] T. Matsukawa *et al.* identified the problem of the citrus taxonomy and related cultivars identification. They developed metabolic fingerprinting method for the identification of the citrus cultivars plants. Metabolic diversity was studied in Citrus, poncircus and fortunella species using HCA; the results suggested that metabolic profiles carry characteristics of hybrid origin and also provide an insight into the phylogenic relationships among citrus species and its cultivars.[54] Christian P. Haas *et al.* have developed automatic digital open-source identification of the chromatographic data analysis for the reaction optimization and screening. chromatographic data remains locked in the vendors softwares and hardware components limiting their potentials in automated workflows and data science applications. In this study an open-source Python project namely MOCCA was developed and the HPLC-DAD data obtained was analysed.[55] Jie Liu identified that *Poria cocos* and *Polyporus umbellatus* are similar medicinal fungi so they developed a fingerprinting

analysis method of monosaccharide and polysaccharide composition by HPLC combined with chemometrics method for characterization and discrimination of the species. The data obtained from the HPLC was further processed by HCA, LDA and PCA. PCA was capable to identify significant differences between the two species, LDA showed a 100 % correct overall classification of the species.[56] Aleksander Lie *et al.* performed multivariate analysis of the elution parameters for RP- HPLC with charged aerosol detection of sucrose monoester regioisomers. The resulting regioisomer retention times were modelled using PLS method and model was iteratively optimized through eliminating insignificant variables effects and recalculation. All the regioisomers of sucrose caprate were successfully identified and assigned and provided sensitivity of 10-100 ng.[57] Pablo Mortera *et al.* developed multivariate analysis method for the quantification of the organic acids in fermented foods. Multivariate calibration analysis coupled with to RP- HPLC with HPLC-DAD was applied to identify and quantify evaluation of short chain organic acids. The data obtained was processed using PARAFAC and U-PLS/RBL for the second order calibration. This improved method was applied to the analysis of many dairy products and wine.[58] Natasa P. Kalogiouri *et al.* progressed the determination method for the bioactive constituents in pistachio nuts for the quality and authenticity of it. Microwave assisted extraction methods were developed for the separation of the phenols and tocopherols, the obtained extracts were analysed using RP-HPLC-UV method. PCA method was used to analyze the differences between the concentration of the bioactive compounds with respect to geographical region, while AHC was used to cluster samples based on their similarity and according to geographical region.[59] W. J. Rogers *et al.* thrived a method for the quality evaluation of the durum wheat. RP-HPLC method along with multivariate analysis to relate the gliadin chromatograms to the quality characteristics of the durum wheat. PCA was used to check relationship between the components. The multivariate analysis of gliadin chromatogram provide means for identifying components important for the quality evaluation of the species.[60] Ya You *et al.* established the spectrum effect relationship between the HPLC fingerprints and *in vitro* antioxidant activity of saffron to improve quality evaluation method of saffron. The fingerprints of 21 batches were collected and assessed, further data obtained was analysed using the SA, PCA, HCA and OPLS-DA. 13 common peaks from 21 batches were selected and some peaks were identified as main active compounds responsible for the antioxidant activity.[61] Umi Kalthom Ahmad *et al.* developed a physical analysis and chemical profiling of the illicit herbal cannabis using multivariate analysis. HPLC method was used to separate cannabinoids in the illicit herbal samples. Profiling of the samples was done using the PCA, results suggested that the samples analysed were obtained from the different geographical region.[62] Dan Gao *et al.* performed the phytochemical analysis of the trifoliate orange during fermentation. The method used for fingerprinting was HPLC-DAD-ESI-MS/MS coupled with multivariate statistical analysis. Total of 8 components were identified and classified according to the HCA and PCA demonstrating fermented and unfermented trifoliate oranges. A reliable PLS -DA method was more suitable for the discrimination of the test samples.[63] Yuvendran muniandy *et al.* enhanced the drug profiling of the cannabis plant species using HPLC method in accordance with chemometrics method. The HPLC separation peaks were obtained and analyzed using CA, SIMCA, PCA to obtain reliable and accurate method for the separation of the cannabis samples.[64] Wei Liu *et al.* aimed to assess the quality of potentilla fruticose sampled from different regions of the china. The method used HPLC fingerprinting method coupled with the suitable chemometrics method. SA, HCA, PCA and DA of the HPLC fingerprints were also performed to provide accurate classification between the varieties of the P.fruticosa.[65] Ke Zhong *et al.* established the HPLC fingerprinting of the *Dendrobium nobile* Lindl. Flower. similarity evaluation combined with CA, PCA, OPLS-DA to evaluate the chromatographic fingerprints of the 15 batches of the flower. The developed method was found to be easy, simple accessible and reproducible for the flower.[66] Xueyang Ren *et al.* developed the method for the rapid discrimination of the *Curcumae rhizhoma* obtained from the three botanical origins. This method utilized HPLC combined with UV spectroscopy along with chemometrics to achieve the aim. 11 components were analysed simultaneously by HPLC. The chemometrics tools such as DT, LDA, SVM and KNN were used to readily and rapidly distinguish the three varieties of the plants. PLS and BP-ANN models were constructed to predict the contents of three chemical components in Curcumae Rhizoma.[67] All these studies are compiled in crisp tabular form for the peers in Table 1.

CONCLUSION

Narrating all of the above examples into explanation, the HPLC-based metabolomics approach is one of the most comprehensive current research tactics for QC of ayurvedic herbal medicines and polyherbal formulations of the Indian ayurvedic medicinal system. The vast biodiversity of plants and the ancient science of Ayurveda give our country, an edge over others, in becoming global leader in holistic healing through herbal medicines. In spite of technological advancement in analytical methods and instruments, standardization of herbal drugs is still an elusive goal, because of variations in the chemical constituents of herbs. These variations may be geographical, seasonal, diurnal or genetic (Chemotypes). In case of polyherbal formulations, further variations are introduced due to adulteration, substitution or omission of an ingredient and also due to batch-to-batch variation in process parameters. Hence, a more scientific and logical HPLC-based metabolomics approach is discussed by considering the entire metabolome of the herb, in order to assess its quality for different variability and authenticity

issues. It is now the high time to regulate the cultivation with the said approach for better QC competences to guarantee the authenticity for ayurvedic herbal medicines and polyherbal formulations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EBN: Edible birds nest; **PDA:** Photo Diode Array; **DAD:** Diode Array Detector; **VWD:** Variable Wavelength Detector; **UV:** Ultraviolet; **CAD:** Charged Aerosol Detector; **ECD:** Electron Capture Detector; **ESI:** Electrospray Ionization; **RID:** Refractive Index Detector; **PCA:** Principal Component Analysis; **HCA/HC:** Hierarchical Cluster Analysis; **OPLS-DA:** Orthogonal Partial Least Squares Discriminant Analysis; **PLS:** Partial Least Square Analysis; **SA:** Similarity Analysis; **PLS-DA:** Partial Least Square Discriminant Analysis; **ATLD:** Alternating Trilinear Decomposition-Assisted Multivariate Curve Resolution; **O-PLS:** Orthogonal Partial Least Square Analysis; **SIMCA:** Soft Independent Modelling of Class Analogies; **DA:** Discriminant Analysis; **KNN:** k-Nearest Neighbours; **PARAFAC:** PARAllel FAC tor analysis; **PLSR:** Partial Least Square Regression; **MLR:** Multiple Linear Regression; **BP-ANNs:** Back Propagation Neural Network Algorithm; **SVM:** Support Vector Machine; **FLR:** Fisher Linear Recognition; **LDA:** Linear Discriminant Analysis; **U-PLS/RBL:** Unfolded Partial Least Square Residual Bilinearization; **AHC:** Agglomerative Hierarchial Clustering; **RMSEC:** Root Mean Square Errors Of Calibration; **RMSEP:** Root Mean Square Errors Of Prediction; **DT:** Decision Tree; **PTR-MS:** Proton Transfer Mass Spectrometry; **PAHs:** Polycyclic Aromatic Hydrocarbons; **MCR-AR:** Mutivariate Curve Resolution with Alternating Regression.

SUMMARY

HPLC-based metabolomics approach is one of the most comprehensive current research tactics for QC of ayurvedic herbal medicines and polyherbal formulations of the Indian ayurvedic medicinal system. To assess the quality of ayurvedic herbal medicines for different variability and legitimacy issues, HPLC-based metabolomics will provide QC competences to guarantee authenticity.

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Cite this article: Patel N, Dave K, Patel R, Vegad K, Kanaki N. HPLC Based Metabolomic Fingerprint Profiling as A Comprehensive Quality Assessment Tool, Why Not for Standardization of Ayurvedic Herbs and Formulations?. Pharmacog Res. 2024;16(4):677-87.