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FINGERPRINTING ANALYSIS AND MARKER COMPOUNDS IN QUALITY ASSESSMENT OF PHYTOPHARMACEUTICALS

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ABSTRACT: Since decades, human beings have been utilizing plants to fulfill their food, medicine, and shelter needs. In the modern era plants, plant-derived products are developed as medicine and drug containing phytoconstituents by combining traditional knowledge and modern technologies. The presence of these phytoconstituents is needed to validate qualitatively and quantitatively to serve the purpose of the development of 'phytopharmaceutical'. To achieve this, explicit plant species, the exact amount of phytoconstituents to show efficacy is required, *i.e.*, quality control of the drug is crucial. Hence, this review focuses on different quality control attributes of herbal material and related products to develop as a 'pharmaceutical drug'. Considering marker compounds and fingerprint analysis as the main characteristic features for maintaining the quality of herbal drugs, this review has been designed. Using these features, falsification is detected in crude drugs, extracts, and final products with the application of different spectroscopic, chromatographic techniques. Thus, this review has quoted the use of reliable, efficient analytical tools through recapitulation of different case studies of adulteration detection of plant material, determination of phytoconstituents present in extracts, and formulations using chromatographic and spectroscopic methods to provide a quality assessment.

INTRODUCTION: The use of plants for fulfilling health needs has been practiced by humans since ancient times. From the last few decades, the use of herbs/phytochemicals has increased for various purposes *i.e.*, in medicine, cosmetics, and nutritional supplements. According to a report by Hexa Research, rapidly growing global market for herbal medicines was estimated at USD 72 billion in 2016 and projected to increase to more than USD 117.02 billion by 2024 [1].

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The correlation between plants and human health supported with modern tools and techniques has opened a new class of plant-derived therapeutics *viz.*, phytopharmaceuticals, polyherbal formulations, dietary supplements [2]. Plants produce various primary and secondary metabolites that can be used in the management of human health. These secondary metabolites are chemical compounds having complex chemistry, belonging to different classes like flavonoids, alkaloids, terpenoids, glycosides, tannins, saponins, lignins, lignans, sterols, *etc*. Most of the time, these are responsible for the efficacy of plants to treat various diseases and disorders. Hence, the determination of content, identification of secondary metabolites in plant parts is necessary using modern tools and techniques for herbal drug development [2].

In recent time, herbal medicines have been gaining popularity due to failure/limited number of treatments from synthetic medicines to find a cure for diseases, for example, Alzheimer's, Parkinson's disease, *etc*. [3, 4] Therefore, researchers are eventually focusing on the treasure of complex chemicals in plants *i.e.* phytochemicals present in plants for drug discovery to find out the best possible treatments. Several therapeutically useful isolates, standardized extracts derived from plants representing a phytopharmaceutical class of drugs that are prescribed by physicians and available in the Indian market [3]. Many plant-derived drugs have a strong influence on drug discovery, one of them is aspirin, a universal medicine; analgesic, and anti-platelet agent, salicin based, derived from Salix spp. and Populus spp., vincristine and vinblastine from *Catharanthus roseus*, taxol *i.e.* paclitaxel from *Taxus brevifolia*; anticancer agents, galantamine from *Galanthus woronowis* for Alzheimer, *etc*. are the few examples of important natural products that have made an impact in medicine [5].

With a view of the increasing use of plant-derived drugs, their quality standards are also needed to maintain. Quality is most important for determining the efficacy and safety of drugs. Indian traditional system of medicines is widely recognized in the universe. In India, various pharmacopoeial standards such as Ayurvedic Pharmacopoeia of India (API), Indian Herbal Pharmacopoeia and Herbal drug monographs in Indian Pharmacopoeia, 2018 gives reliable quality attribute to check the authenticity of ayurvedic formulations, plant-derived medicines, crude plant drugs, herbal extracts, essential oils as per the Drugs and Cosmetics Act 1940 [6]. Indian Pharmacopoeia (IP) 2018 contains around 165 monographs of essential oils, crude herbs, and herbal extracts [7].

Ayurvedic Pharmacopoeia of India (API) is published in two parts. Part I has eight volumes containing monographs of single herbs containing 514 medicinal plants, 5 plant/animal/insect derived products and 21 metal and minerals, and about 105 hydro-alcoholic and water extracts. Whereas its Part II has four volumes and included 202 monographs of ayurvedic formulations, according to Ayurveda Publication, Ministry of AYUSH, Government of India, 2018 [8]. Use of microscopic, various spectroscopic, chromatographic techniques such as ultraviolet-visible (UV-visible) spectrophotometer, high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) for assay, identification tests in individual monograph has included in pharmacopoeial standards. Medicinal Plant Division of Indian Council of Medical Research (ICMR) has developed standard reference series as "Quality standards of Indian Medicinal Plants" of total 17 volumes [9]. About 580 monographs of medicinal plants are published in it. It has covered detailed microscopic, macroscopic descriptions of plant parts that are used as a drug, its colored photographs. Also other quality control parameters and chromatographic fingerprint profile along with information on pharmacological use, clinical studies, and toxicity in few cases has been included in it. Along with this, in another publication by ICMR titled as "Phytochemical Reference Standards of Selected Indian Medicinal Plants", monographs on 120 marker compounds from frequently used medicinal plants are included. It contains modified isolation procedures of phytochemical reference standards, their quantification in the crude extract by HPLC, with special emphasis on structural characterization of a purified marker and its physical and spectral data using modern spectroscopic methods like UV-Visible, infrared (IR), nuclear magnetic resonance (NMR) is published [9].

Phytopharmaceuticals as a New Avenue: Phyto means 'plant-based, according to gazette notification of CDSCO [10], “phytopharmaceutical drug” is defined as a purified and standardized fraction with a defined minimum four bioactive or phytochemical compounds (qualitatively and quantitatively assessed) of an extract of a medicinal part or its part for internal or external use of human beings or animals for diagnosis, treatment, mitigation, or prevention of any disease or disorder but does not include administration by parenteral route." Phyto-pharmaceuticals or plant-based medicines are different from synthetic drugs in terms of the presence of active constituents present in them. These are a complex mixture of chemical substances which are pharmacologically active. Herbal medicine contains either a single herb or a combination of herbs. Active constituents from raw herbal material are extracted using solvents of different polarities.

The new phytopharmaceuticals regulation permits the development of drug development using advanced techniques of solvent extraction, fractionation, modern formulation development, *etc*. [10]

Quality Assessment: Any herbal product rather any compound to pass as a drug need to go through scientific validation which includes qualitative and quantitative assessments of substances present in it. Optimum level of therapeutically active components is necessary and this can be achieved by using certain modern tools and techniques which analyze these compounds in a mixture. There is steep growth in 'regulated phyto-pharmaceuticals', which comply with rules and regulations of governing bodies, here quality control and standardization are necessary to be achieved, and for this, modern tools and techniques play a crucial role. As these plant materials are mainly sourced from their natural habitats from different geographic regions, they affect the chemical profile (level and concentration of phytoconstituents present) of the same herb. Along with this erroneous identification, accidental or intentional adulterations are other factors that affect the quality the efficacy of herbal material. Hence there is a need to develop quality control methods that can ultimately ensure the efficacy of the medicine. The quality of herbal medicine is ensured by its true identity, purity, exact amount of biologically active secondary metabolites. This has an impact on its efficacy and safety. Factors that cause a variable amount of secondary metabolites are mentioned below, which are considered in quality assessment [11].

- Seasonal variations
- Growing conditions (wild) or cultivation
- Stress
- Harvesting and post-harvesting process
- Time of collection
- Storage conditions maintained after collection
- Extraction methods used
- Parts used in phytomedicine
- Adulteration/substitution of raw material
- Age of plant

In the case of plant material, morphology, macroscopy, microscopy with specific chemical tests; specific for groups of phytoconstituents, are performed to authenticate or qualify the sample. For the detection of chemical composition analytical techniques are developed. These give a complete chemical profile of herbs, their extracts, and phytopharmaceuticals by generating certain patterns of plant material according to the composition of secondary metabolites. These patterns are called 'fingerprint profiles', which are the identity of specific plant material, formulation, *etc*. So in this review significance of fingerprinting analysis and marker-based validation has been highlighted through various case studies of adulteration of plant material, analysis of polyherbal formulations as the challenge in quality control and development of medicinal plant preparations, focusing on the reliable use of different analytical tools and techniques.

Marker Based Standardization: Marker is a chemically defined constituent or group of constituents in herbal preparation or herbal medicinal product which serves the purpose of quality assessment. It is indicative of the quality of herbal medicine, crude drug as it is quantified, and to some extent, the limit for its presence is determined in the herbal drug. As any substance in higher quantity can cause toxic, adverse effects and if in lower quantity it could not show expected therapeutic effect, therefore, it should be present in optimum quantity. According to EMA's reflection paper, 2008 on markers used for qualitative and quantitative analysis of herbal medicinal products [12] markers are classified into active and analytical markers. Active markers are the chemicals that are those who contribute to therapeutic activity and not fully responsible for whole therapeutic activity, and analytical markers serve only an analytical purpose. EMEA's herbal quality guidelines 2018 [13] say that where the herbal medicinal product contains herbal substances and/or herbal preparations with ‘constituents with known therapeutic activity’, then markers should be used for identification and quantification. Several markers are qualitatively and quantitatively examined using different analytical procedures. However, studying only marker compounds does not suffice the whole quality control of medicinal plants, herbal products as there are many common markers like rutin, quercetin, gallic acid, *etc*. are present in the majority of plants, and plants lack unique chemical compounds. Also, just studying marker compounds

does not reflect the complex structure of plant metabolites. To overcome this, fingerprinting analysis is superior which elaborates on the complex nature of herbal medicines.

Fingerprinting Analysis: Quality assessment and standardization are important to protect the integrity of herbal products to be used as a pharmaceutical drug. As herbal drug contain myriad of compounds which creates challenges in establishing quality control standards for raw materials and finished products. Therapeutic efficacy is based on the complex interaction of these constituents hence, in this case, a pattern-oriented approach; 'fingerprinting' is used. It not only evaluates the presence or absence of desired markers or active constituents but also gives the complete set of ratios of all detectable analytes present [14, 15].

It can be obtained by spectroscopic, electrophoretic, and chromatographic techniques. A fingerprinting is a characteristic profile of plant material that reflects its chemical composition. In herbal drug development quality of raw material, processed extracts, finished products are monitored, chemically evaluated for their quality using fingerprint analysis. Along with morphology, microscopy, and macroscopy fingerprinting analysis gives a lot of information about the chemistry of plant material. It is used as a qualitative and quantitative measure for monitoring the quality of the herbal drug. In some cases, we cannot identify drugs through classical microscopy and macroscopy like in the case of Talispatra (*Abies webbiana*) which is adulterated/ substituted with English yew (*Taxus baccata*) [16]. In such cases, fingerprinting analysis overcome these difficulties. Different fingerprinting methods are used to detect adulteration in herbal crude drug, extracts, or finished product containing a mixture of herbs along with identification, differentiate between closely related plant species.

Analytical Tools and Techniques Used:

Spectroscopy: Spectroscopic techniques such as ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS) are important tools that are generally used. These simple techniques give ample information about the chemistry of phytochemicals present in herbal drug or phytopharmaceutical drug and hence widely used. Spectroscopic techniques are simple, fast, cheap and non-destructive and mainly it does not need sample preparation which makes them easy for detection of falsification of plant material [17].

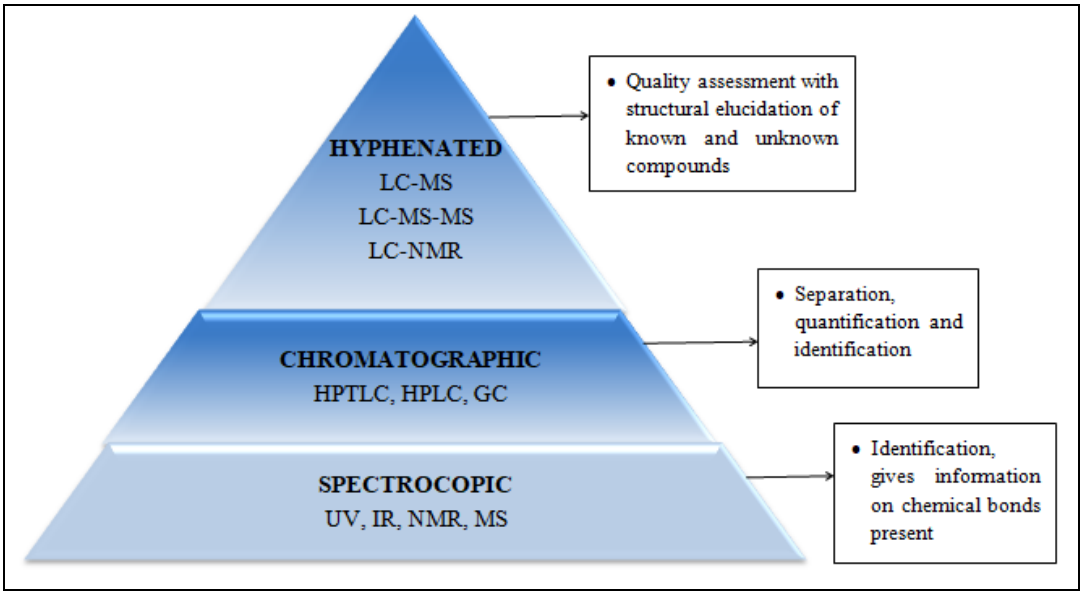


FIG. 1: HIERARCHY OF ANALYTICAL TOOLS AND TECHNIQUES

IR spectroscopy has gained popularity in the identification and authentication of plant-based material and related products. Interaction between electromagnetic radiations in the infrared region and sample gives unique patterns *i.e.*, spectra of bands and overtones, and gives a path to conclude the chemical structure of constituents present in plant material. As it is simple, non-destructive,

rapid, does not need a solvent, hence becoming popular in herbal, plant-based material identification. All types of bonds, functional groups show special absorption bands which facilitate the structure determination of the compound. IR region ranges from 0.8 - 200µ i.e. 12500 cm^{-1} - 50 cm^{-1}. It is divided into Near IR (NIR) (0.8-2.5 µ), Mid IR (2.5-15µ, most important region), and Far IR (15-200 µ). Among the functional group regions in IR the region below 1500 cm^{-1} called as fingerprint region, it plays an important role in analyzing two compounds containing the same functional groups, which shows the same bands above 1500 cm^{-1} but shows different characteristic features, which enable proper identification and differentiation [18].

In whole IR regions, previously less explored or neglected, NIR spectroscopy has become a dominant tool nowadays in identifying, detecting fraudulence in plant material, herbal supplements, polyherbal formulation and food products [19, 20]. NIR spectroscopy with a spectral range from 780 nm to 2500 nm can reveal much complex information depending on the bands obtained by the combination of overtones and fundamental vibrations of specific functional groups like O-H, C-H, S-H, and N-H through a molecular bond. The NIR spectrum reflects the responses of the overtones and higher vibrations of the molecular bonds in the sample under study [21]. Nuclear Magnetic Resonance (NMR) is a powerful technique that is useful in the structural elucidation of compounds. It gives information on the chemical nature of compounds. Mass spectrometry is one more technique in which structural determination of known and unknown compounds is done qualitatively and quantitatively by obtained mass fragmentation patterns of samples [22].

Chromatography: Chromatography is the separation technique that separates the components of a complex mixture, identifies them, and analyzes qualitatively and quantitatively using mobile phase and stationary phase. As the herbal is being complexed in nature, this versatile system is effective in the case of herbal products to obtain 'unique chemical fingerprint' of plant material and phyto based medicine as a quality control measure. TLC and HPTLC are used for decades and till now proven to be powerful tools for analysis. Small volumes of the mobile phase, the number of scans of the developed plate, simultaneous analysis of components in the mixture are some advantages of HPTLC [23].

After HPTLC, one majorly used technique is HPLC, with its pros such as better separation efficacy, a more sensitive detector and more prominent peaks than HPTLC. Use of different types of detectors like Ultraviolet (UV), photodiode array (PDA), refractive index (RI), and infrared (IR) makes it feasible for a wide number of substances. Capillary HPLC and ultra-performance liquid chromatography (UPLC) have increased analysis efficiency hence; HPLC is being replaced by UPLC as it has augmented speed, separation efficiency, and sensitivity. A higher pressure range of about 6000-15,000 psi has shortened analysis time [24]. While another form of chromatography, such as Gas chromatography (GC) is useful in volatile components analysis. As the plant material contains volatile oil hence, GC becomes superior in this case. Capillary columns give high selectivity for the simultaneous separation of volatile components within a shorter time. However, the analysis of polar, non-volatile, and heat-sensitive components is cannot be done. This is the limitation of GC. As data/ peak analysis is database-driven, it is possible to detect and identify unknown compounds in a complex mixture [22, 25].

Electrophoretic Techniques: Electrophoresis is the technique in which separation is based upon the mobility of charged particles under the electric field. Capillary electrophoresis is the technique in which separation takes place on micro-column based upon the mobility of the molecules in the electric field. Its advantages include short analysis time, low solvent consumption, and increased separation efficacy [26].

Capillary electrophoresis has various types, which includes

- Capillary Zone Electrophoresis (CZE)
- Capillary Isotachophoresis (CITP)
- Capillary Isoelectric Focusing (CIEF)
- Micellar Electrokinetic Capillary Chromatography (CEKC)
- Capillary Gel Electrophoresis (CGE)
- Capillary Electroosmotic Chromatography (CEC)

Hyphenated Techniques: Hyphenation is nothing but the combination of two methods, as only one method cannot fulfill the analytical requirement of a complex mixture; hence it is combined with another method to get more information about the complex nature of herbal medicine. It is coupling of chromatographic techniques with online spectroscopic techniques [25]. It gives multicomponent data that facilitates the capture of more knowledge *i.e.* chemical, physical, structural information about secondary metabolites present in plant extracts or fractions. Liquid chromatography (LC) is hyphenated with PDA, MS, TOF/MS, Q/TOF/MS, and even two or three techniques are combined. The higher speed of liquid chromatographic techniques such as HPLC, UPLC, UHPLC give fingerprint pattern, and spectroscopic techniques provide structural information which facilitates analysis and identification process in the same line. For example, a simple coupling of gas chromatography (GC) with mass spectrometry (MS) *i.e.* GC-MS where, GC is useful for analyzing small and volatile compounds whereas MS determines the molecular weight and give fragmentation pattern of a sample, which is then compared with an online library for identification of the compounds present in the mixture. LC-MS, GC-MS, LC-NMR are majorly used for qualitative and quantitative analysis. Hyphenation with mass spectrometry (MS) gives exact molecular mass, fragmentation pattern through which structure is depicted and NMR provides confirmation and position of atoms present, which confirms the structural features and aids in the exact identification of unknown compounds. Hyphenation technique is not limited to the use of single spectroscopic techniques but more than two are also combined *i.e.* LC-MS-MS, LC-MS-NMR, *etc*. [27, 28]

Chemometric Analysis: Nowadays, the use of chemometric analysis has increased, which has certain advantages in maintaining and interpreting the data obtained from spectroscopic and chromatographic techniques. In a chemometric analysis, integration of mathematics and statistics can offer methods for chemical measurements and provide new approaches for analysis of data obtained from spectroscopic, chromatographic, and chemical analysis. In a quality evaluation of medicinal plants or herbal medicines, it can be used to optimize experimental methods, pretreatment of fingerprint, spectra, extract maximum useful information, and analyze results. There are different methods in a chemometric analysis like Principle component analysis (PCA), Hierarchical Cluster Analysis (HCA), Pattern recognition, Discriminate analysis (DA), *etc*. that develop models through which data is analyzed [14].

Case Studies: Following are the different case studies that represent the spectroscopic and chromatographic methods in fingerprinting analysis and marker-based identification as a strong and reliable tool in the quality assessment of the herbal drug.

Marker-based quality control profiling of *Trifolium pretense* L. was developed by Oza M J *et al.,* [29] *Trifolium pretense* L. is also known as 'Red clover', belonging to family Fabaceae. It is a biennial or perennial villous forage important herb. Its flowering tops are used as dietary supplements worldwide by females as a source of phytoestrogen. It is indigenous to central and southwestern Asia. Worldwide it is distributed in Europe, North Africa, China, Australia, and northern India. It is used in the form of herbal tea or liquid, capsules of isoflavone-rich extract. It mainly contains isoflavone. Its leaves contain higher percentage of isoflavones than flowers, stems, and roots. In this study, HPTLC fingerprinting of ethanolic extract of leaves, flowers, stem, and roots was done using chloroform: methanol (9:1 v/v) at 254 nm on a precoated silica Gel 60 GF254 plate. A rapid, precise HPTLC method was developed for the ethanolic extract of leaves, stem, flower, and root; which separated the phytoconstituents in extracts and quantified the two marker compounds formononetin and biochanin A, as a quality control standard for *Trifolium pretense* L.

HPLC is another chromatographic technique that is widely used in phytopharmaceutical development. Choudhary S *et al*., have developed a rapid, simple HPLC method for estimating marker compounds in a mixture of extracts containing three medhya rasayanas *Convolvulus pluricaulis*, *Withania somnifera*, *Bacopa monnieri*. Bioactive marker compounds of these plants *viz*., scopoletin, withaferin A, bacoside A 3, bacopaside II, jujubogenin, and bacosaponin C were simultaneously estimated first time and 20 min run time,

and low amount of solvents makes it eco-friendly. So in this method, after various trials with column and solvent systems, the separation was done on Water X Bridge shield with BEH technology (2.5 µm, 4.6 × 75 mm) using ACN: 0.2 % OPA in the water at gradient mode. All the markers in the mixture were well resolved from each other. Further, it was validated according to ICH guidelines. This is an example where HPLC has shown its use in the simultaneous detection of bioactive phytoconstituents in a complex mixture. This method is further applied to quality control, standardization of different herbal formulations of *Convolvulus pluricaulis*, *Withania somnifera*, and *Bacopa monnieri* [30].

Shaikh S and co-workers developed a simple RP-HPLC method for simultaneous detection of ellagic acid, quercetin, and piperine in two commercial ayurvedic formulations of dental care powder, *i.e.*, Vithoba and Dabur. These are used to maintain oral hygiene, indicated for various dental problems, and claim to make stronger teeth and gums. The selected formulations were containing *Terminalia bellirica* (Combretaceae), *Piper nigrum* (Piperaceae), *Terminalia chebula* (Combretaceae) and other crude drugs [31].

In the traditional Chinese System of Medicine stem of *Dendrobium officinale* have high medicinal value but its limited supply makes it costly and therefore this is subjected to adulteration with morphologically same *Dendrobium devonianum* in the herbal market in China. Ye Zi *et al*., developed rapid and reliable HPLC and HPTLC fingerprinting which could differentiate two species based upon their phytochemical content. HPLC fingerprinting exhibited 15 common peaks, but the chromatographic pattern showed a high difference between 20-40 min. Thus, HPLC fingerprinting can serve as an efficient tool to differentiate. Also, structural elucidation of about 26 phytochemicals present was done by HPLC-ESI-MS. Among those, violanthin and schaftoside were used as chemical marker compounds. Violanthin was found only in *Dendrobium offinale*, whereas schaftoside was common in both. Hence, adulteration of the stem of *Dendrobium officinale* with *Dendrobium devonianum* can be detected. Thus this experiment identified the specific marker compound that differentiated the stem of two species using fast, reliable HPLC, HPTLC method, which gave a quality control strategy for both herbs [32]. *Ginkgo biloba* is one of the widely used herbs as it has a range of therapeutic applications like anti-diabetic, anti-hyperlipidemic, antihypertensive.

It has promising effects in treating epilepsy, dementia. It is used as a supplement by a large population, but these marketed samples is frequently adulterated; do not contain authentic plant material. A study conducted by Frommenwiler D. A. *et al*., [33] revealed the use of comprehensive HPTLC fingerprinting for purity analysis of commercial products of *Ginkgo biloba*. Total 59 products analyzed in the study were tablets, capsules, soft gel capsules sold as food supplements or medicine.

The developed HPTLC method could identify the adulteration using peak profile generated from chromatogram images prior and after derivatization, also evaluated the limits of rutin and quercetin along with other adulterants like sophora fruit, sophora flower bud, buckwheat herb. The developed HPTLC method showed certain specific observations like intense quenching zones at a position of rutin under 254 nm, which were absent in authentic *Ginkgo biloba*.

Whereas, before derivatization, a blue fluorescent zone at 366 nm of chlorogenic acid, which is characteristic of *Ginkgo biloba* was observed. Along with these, an additional blue zone between R_f 0.6 -0.7, green zones between R_f 0.2-0.3 which considered due to the high level of quercetin in a few products. Other types of analysis that were performed were limit tests for rutin and quercetin, quantification of flavonol glycosides as per specifications of USP. It was observed that products compliant with total flavonoid assay had weak intensity in HPTLC fingerprints. Overall only 11 products out of 59, complied with USP specifications for identi-fication, limit detection of rutin and quercetin, flavonoid content. This was the largest study performed on commercial compounds and proved the ability of HPTLC in providing information concerning the raw material acquisition, product development, intermediate steps in manufacturing, and final product compliance. Thus, this HPTLC study highlighted poor practice within the industry.

Alpinia galangal, which is used as 'rasayana' belongs to family Zingiberaceae. It is medicinally important in ayurvedic system of medicine to cure ailments like rheumatism, fever, bronchial catarrh, stomach pain. It is known as kulinjan in the Indian herbal market. It is reported to have antimicrobial, anticancer, antidiabetic, anti-HIV activities. It contains bioactive chemicals which include its main flavonol compound known as galangin. As it has high demand in the market it is seen that it is adulterated with two other species *viz*. *Alpinia calcarata* and *Alpinia officinarum* [34].

Upadhye S and co-workers [34] have developed a simple marker-based HPTLC method for the identification of correct species to overcome this problem. In this, galangin was used as a marker compound, and it was quantified in methanolic extracts of rhizomes of three plant species. Galangin was found to contain in all species in variable amounts. The quantitative analysis showed the highest amount of 7.67 ± 0.36 mg/g in *Alpinia galangal*, 5.77 ± 0.71 mg/g in *Alpinia officinarum*, and 4.31 ± 0.44 mg/g in *Alpinia calcarata*. The developed method was validated as per ICH guidelines. Thus, this study proved the use of HPTLC in herbal raw material identification.

Bosewellia serrata is well known medicinal herb in the traditional system of medicine known for its therapeutic effects. Oleo-gum-resin exudes from the bark of this tree are called Indian frankincense, which has been used in Asia and African folk medicine for arthritis. Katragunta K *et al*., [35] have developed UPLC-PDA method for the quantification of six markers *viz*. keto boswellic acid, 3-O-Acetyl-11-keto β-boswellic acid, α-Boswellic acid, β-Boswellic acid, 3-O-Acetyl-α-boswellic acid, and 3-O-Acetyl-β-boswellic acid from crude resin as their quantity defines the quality of the resin. Methanolic extract of crude resin was subjected to UPLC-QToF-MS/MS system in which about 16 triterpenoids were structurally characterized. Resin samples and commercially available formulations (in the form of a capsule, tablet, powder, gel, and tea infusion) were analyzed, containing both single and polyherbal formulations. Indian and European Pharmacopoeial guidelines limit the content of 3-O-Acetyl-11-keto-β-boswellic acid, 3-O-Acetyl-β-boswellic acid as 1% w/w on a dried basis as an active principle in herbal preparations containing *Boswellia serrate*. In this study, markers were found in the range of 0.14-16.08% in all commercial samples. Out of 14 samples tested, only 4 samples could pass the pharmacopoeial standards; from this, it can be concluded that it is necessary to scrutinize the herbal product formulations for standardization.

Synthetic compounds, illegal pharmaceutical ingredients are often added to herbal supplements, phytotherapeutic formulations. These compounds can cause life-threatening events. There is a huge demand of herbal supplements for weight loss worldwide which are found to contain corticosteroids and other active pharmaceutical ingredients of different classes such as anxiolytics, anti-depressants, laxatives, anorexics, *etc*. [36, 37]. FTIR was found to be useful in the detection of such synthetic substances in herbal slimming powders containing caffeine, modafinil, phenolphthalein, and salbutamol. Khare B *et al*., [38] screened 20 samples of such powders by FTIR analysis. Comparison and overlay of sample spectrum with a standard spectrum which showed similarities in absorption spectra and detection, was made. In these, about seven samples were found to be adulterated. Out of seven, two samples were found to contain two adulterants, and another five were found to contain one adulterant. Thus, this simple study revealed the efficacy of FTIR in the qualitative evaluation of herbal supplements to identify adulterants in it.

Saffron is one of the costly and unique spices which are highly subjected to fraudulence. Commonly it is mixed with petals of calendula flower, powdered rhizomes of turmeric, fruit extract of gardenia, flowers of buddleia (butterfly bush), which contains pigments similar to esters (crocetin esters) present in the saffron. Ordoudi *et al*., [39] detected the presence of substances that mimic the saffron in appearance like synthetic dyes. In this study, a commercial sample of saffron was compared against the standard authenticated sample. The initial colorimetric analysis showed that the sample is not pure; similarly, UV-Visible spectroscopic examination of the aqueous extract showed specific absorbance value at 440, 257, and 330 nm. Among these, $E_{\%257}$ was a higher value reported for high-quality standard saffron samples;

also, $E_{\%330}$ was slightly below the upper limit of standard value. These findings showed the importance of recording the UV-Visible spectrum for purity identification. Further HPLC analysis showed the absence of main component picrocrocin in the commercial sample. Derivative spectroscopy detected the presence of artificial colorants and identified known colorants as tartrazine and sunset yellow by FTIR and NMR analysis. Additionally, other components such as propane-1,2-diol, propan-2-ol, triacetin, and free saturated fatty acids were detected. Hence, the effectiveness of a multidisciplinary analytical approach (HPLC, UV-Vis, mid-infrared (FTIR), and nuclear magnetic resonance (NMR)) was proven in this. Thus, one can identify such type of falsification through the mentioned UV-Visible method only.

Celoy cinnamon; true cinnamon, inner bark of *Cinnamon zeylanicum* belonging to family Lauraceae, is widely used in medicines and cosmetics. *Cinnamon zeylanicum* is substituted/adulterated with *Cinnamon cassia* as it is expensive than *Cinnamon cassia*. Morphologically Cassia can be distinguished but in the case of powder, it is hard to determine adulteration. To address this issue, Yasmin J *et al*., [40] have employed FTIR and NIR spectroscopic method with chemometric analysis to detect true cinnamon and cassia cinnamon powder with various concentrations (5-50% wt.) of an adulterant. A total of 195 samples were analyzed, containing true cinnamon, cassia, and adulterated samples. Spectral data were reprocessed with a spectral reprocessing method of standard normal variables (SNV), Savotzky-Golay derivatives, and four-point smoothing was applied. The independent component analysis plot showed a spectral peak difference between true cinnamon powder and mixture for both NIR and FTIR. Informative spectral information to differentiate was observed between 4000-5400 cm^{-1} for NIR and 675-1800 cm^{-1} for FTIR, which showed unique peaks.

Saraca asoca is a medicinally important tree of the Indian system of medicine; its bark has a therapeutic value against gynecological problems. Its morphological similarity with *Polyalthia longifolia* (false Ashoka) raises the concern of adulteration and substitution. Sharma P *et al.,* [41] performed chemical and genetic fingerprinting of each plant and to develop the tool which can be applied as a better identifier of *Saraca asoca*. HPTLC was used to develop chemical fingerprint, whereas genetic profiling was based on the Amplified fragment length polymorphism (AFLP) technique using six primers. Stem bark samples of each plant were collected from various geographical locations. Phytochemical analysis was done in hexane, ethyl acetate, and methanol extracts by the successive solvent extraction method. HPTLC plates were developed, subsequently scanned on three different wavelengths. Statistical analysis of the chemical data, obtained by analyzing the three different solvent extracts individually, in comparison to the methanol and the ethyl acetate extracts, chemical data from the hexane extract showed tight clustering of the *Saraca asoca*. Hexane extracts found to be versatile than other extracts to generate signature HPTLC fingerprints for the *Saraca asoca*, which can differentiate it from the most potent substitute *Polyalthia longifolia*. Statistical analysis revealed 98.6% of polymorphism between *Saraca asoca* and *Polyalthia longifolia* based on the chemical data, whereas 97.1% polymorphism was obtained using genetic fingerprinting.

More discrete and reliable clustering was offered by the chemical analysis rather than the amplified fragment length polymorphism (AFLP) as genetic diversity in *Polyalthia longifolia* caused a distributed pattern of clustering, which was interrupting the absolute identification of the original. Thus, in this case, chemical fingerprinting has proved as a better classifier than a genetic one, still genetic method can be integrated and used as the supporting tool.

Boerhavia diffusa, commonly known as Punarnava, belonging to the family Nyctaginaceae, is an important medicinal plant of traditional Indian medicines. Its roots and the whole plant are used. It is adulterated with its morphological similar plant *Boerhavia erecta* from related species. Although they have morphological similarities that can be distinguished from botanical characters, their above-ground parts are sold in powder form in the Indian herbal market; therefore, it is difficult to distinguish in powdered form. Hence, Vadivel V and Brindha P [42] have developed a chemical fingerprint profile of whole plant powder extract of both plants for differentiation. Powdered material

was extracted in chloroform and methanol using orbital shaking at 500 rpm for 3 h. Further, chloroform extract was analyzed by NMR, and methanol extract was analyzed by UV, IR, HPLC and HPTLC to get chemical fingerprints for differentiation. Methanol extract could not analyze in NMR. In a UV visible scan of methanol extract of *Boerhavia diffusa*, maximum absorbance was observed at 212, 362, 428, 663 nm whereas in *Boerhavia erecta* absorbance was observed at 214, 308, 349, 532 663 nm. Similarly, in FTIR analysis different peak patterns in the fingerprint region i.e. 400-1000 cm^{-1} for two plants indicated differentiation. These UV and IR profiles can be applied to authenticate both the plants. Further, the HPTLC fingerprint of *Boerhavia diffusa* exhibited nine peaks whereas twelve peaks were observed in *Boerhavia erecta*. In HPLC analysis of five peaks were observed for *Boerhavia diffusa* with unique peaks at 3.4 and 5 min, whereas for *Boerhavia erecta* four peaks were observed with a remarkable unique peak at 3.6 min. Different peaks in the NMR spectrum of chloroform extract of both plants were observed; hence it can be useful to differentiate. Therefore, all analytical fingerprint profiles of both plants made differentiation in this study and this is useful to detect, prevent adulteration of genuine plant *i.e.*, Punarnava from its adulterant. Also, this can be applied to the quality control of both plants.

Curcuma longa i.e. turmeric rhizome is one of the main widely used herbs in many formulations, cosmaceuticals, and as a spice. Its main phyto-constituents, curcuminoids are curcumin (CC), desmethoxycurcumin (DC), and bisdemethoxy-curcumin (BC) accounts 3-5 % in turmeric extracts. Pharmacologically curcuminoids have a wide array of actions like anticancer, antibacterial, anti-Alzheimer, *etc*. These are photosensitive, which get degraded during the manufacturing process, extraction processes, and improper storage conditions. Vanillic acid (VA), vanillin (VN), ferulic acid (FA), and 4-hydroxybenzaldehyde (HB) are some of its degradation products. Various HPLC, HPTLC methods were developed for the determination of curcuminoids, but the simul-taneous estimation of curcuminoids and their degradation products could not be possible using chromatographic techniques. Anubala S *et al*., [43] developed non-aqueous capillary electrophoresis (NACE) technique which was found to be efficient for the simultaneous separation of curcuminoids with their degradation products. In this, turmeric powder, turmeric capsule, turmeric milk, and lenticels (in which turmeric was used as a colorant to give a better appearance) were analyzed.

Ultrasonication extraction and ultrasonication-assisted phase separation extraction methods were used for the extraction of targeted compounds from the tested samples. The separation was achieved in a non-aqueous background electrolyte (NA-BGE) mixture of 12.5 mM sodium tetraborate (STB) in 0.1 sodium hydroxide, methanol, and 1-propanol at separation voltage of 20kV (negative polarity) in a capillary of 70 cm length. The degradation compounds found in the herbal products were in the range of 0.53–2.24%. Hence, simple, fast NACE was developed, which was found to be beneficial for the separation of lipophilic components in electrolyte mixture with better separation of targeted compounds with direct injection of acetonitrile extract. Further, this method can be coupled to MS for the analysis of unknown compounds.

Guggul tree, *Commiphora wightii* is one of the most important and used medicinal plants, belonging to the family Burseraceae. Oleogum resin obtained from this plant has various pharmacological effects like anti-hypertensive, hypolipidaemic, *etc*., and its extract is available in tablet, capsule form. It has become an endangered plant species in Rajasthan, India [44]. Due to its supply and demand gap, the authentic resin is subjected to adulteration by gum resin obtained from different Commiphora species and other plant species. To detect this adulteration of guggul, Ahmed R *et al.*, [45] have developed HPLC chromatographic fingerprinting method using twelve phytoconstituents of different chemical classes such as sterol, terpenes, lignin, and quantified E and Z-Guggulsterones; the two marker compounds. Twenty-two authentic and commercial samples of oleogum resin of *Commiphora wightii* and nine oleo gum resin samples from *Mangifera indica*, *Azadirachta indica*, *Commiphora myrrha*, *Acacia nilotica*; possible adulterants were collected from India and Pakistan and some from the USA. Out of all, one commercial sample was ethyl acetate extract of *Commiphora wightii*. In their previous study, researchers isolated twelve standard

compounds from commercial guggul samples (ethyl acetate extract), and the same was used to develop chemical fingerprint. The isolated standard compounds were 8β-hydroxypregnene-4,6-diene-3,20-dione, diasesartemin, sesamin, 20(S)-acetyloxy-4-pregnene-3,16-dione, E-guggulsterone, Z-guggulsterone, guggulsterol III, 5-(11′Z-heptadecenyl) resorcinol, (13E,17E,21E)-polypodo-13, 17, 21-trien-3,8-diol, (13E,17E,21E)-8-hydroxypolypodo-13,17,21-trien-3-one, mangiferolic acid , and 5-(13′Z-nanodecenyl) resorcinol.

HPLC-UV fingerprints of methanolic extracts of all samples and ethyl acetate extract were compared with the chromatogram of a mixture of isolated standard compounds. LC-UV analysis was carried out at 243 nm and 205 nm, owing to the detection of compounds, respectively. Fingerprints of 5 selected samples of gum resin of *Commiphora wightii* as mentioned in the paper, revealed the presence of nine standard compounds in two resin samples, whereas eight standard compounds in three samples.

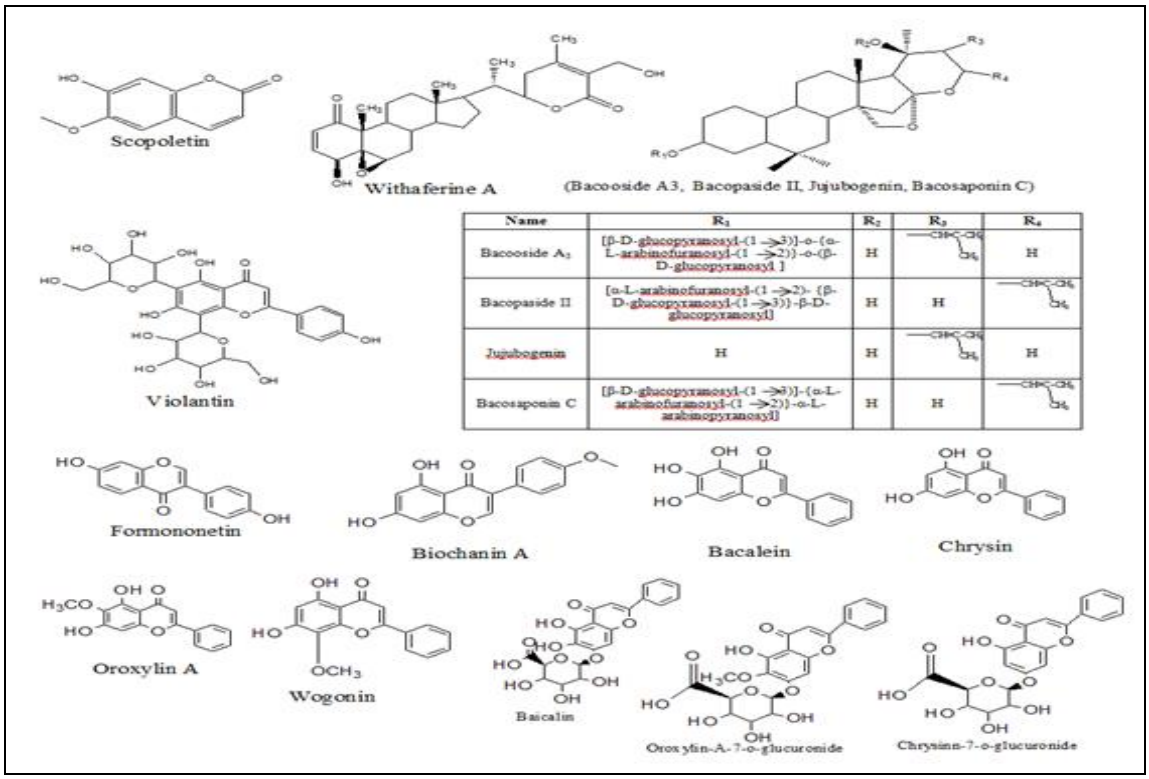
In all resin samples of *Commiphora wightii* E-guggulsterone, Z-guggulsterone, (13E, 17E, 21E)-ploypodo-13, 17, 21-trien-3,8-diol and (13E, 17E, 21E)- 8- hydroxypolypodo-13, 17, 21-trien-3-one these four marker compounds were found to be present. Whereas, 5-(11′Z-heptadecenyl) resorcinol, mangiferolic acid, 5-(13′Z-nanodecenyl) resorcinol oleogum resin related to *Mangifera indica* found to be present in commercial ethyl acetate extract. This indicated the adulteration of commercial samples with *Mangifera indica*. No standard compound was detected in chromatograms of gum resins of *Acacia nilotica*, *Aazadirachta indica*, and *Commiphora myrrha*. Further, the developed fingerprinting method was validated for quantification of E and Z-guggulsterone in all of the *Commiphora wightii* gum resin samples, and their percentage content was found in a range of 0.051% to 0.867% (E-guggulsterone) and 0.063% to 4.623% (Z-guggulsterone) respectively. It was observed that the content of Z-guggulsterone was more than E-guggulsterone in the majority of samples. Thus, the developed HPLC-UV fingerprint profile effectively detected adulteration in an authentic sample and it can be used to give authenticity to raw herb material and can be used in the analysis of phytoformulation of guggul.

As an alternative to conventional methods, capillary electrophoresis has gained popularity with its advantages. A study conducted by Gomes AF and co-workers [46] on *Lippia alba*, a medicinal plant widely used in different regions of central and South America as a tranquilizer and for gastro-intestinal disorders. In this, the micellar electro-kinetic capillary electrophoresis (MECK) method was developed for analyzing and quantifying marker compounds present in the methanolic leaf extract of *Lippa alba*.

Along with electrophoresis, for comparison HPLC-DAD method was developed. About six marker compounds *viz*., acteoside, geneposidic acid, 8-epi-loganin, mussaenoside, chrysoeriol- 7- O-diglucuronide, and tricin-7-O-diglucuronide were separated, extracted and quantified. These neutral flavonoids and glycosides in the extract were separated by micellar electro-kinetic capillary electrophoresis in 25 min using 75 mm sodium dodecyl sulfate (SDS) in borate buffer made with 50 mM sodium tetraborate solution (pH 9.5) and 5% isopropanol with 20 kV voltage at 25 ℃, 254 nm. SDS is used as a surfactant that forms pseudo stationary phase and facilitates separation between hydrophobic micelles and hydrophilic buffer to enhance separation. In the case of the HPLC method, the detection was performed at 240, 254, 325 and 350 nm wavelength on Pro C18 RS column (150 × 4.6, 3 µm particle size) at 45 ℃, at a flow rate of 0.9ml/min, and 2.5 µl of the sample were injected. A mixture of 0.02% (v/v) trifluoro-acetic acid in water and CH_3OH/ CH_3CN (3:7) in gradient mode was used as a mobile phase. If we compare both the developed methods, the electrophoresis method could separate marker compounds in 25 min, which was less than half time required for HPLC *i.e.* 60 min. The two isomers, acteoside, and isoacteoside were resolved by HPLC but not by the capillary electrophoresis method. They were quantified as total phenylpropanoids in electrophoresis analysis. Both the methods gave approximately the same quantification of all compounds present. Methods were validated according to ANVISA and ICH guidelines.

Hence, capillary electrophoresis was found to be a valuable alternative to conventional methods; economically and environmentally.

Andrographis paniculata; Kalmegh is one of the most important plants having main wide therapeutic activities like antidiabetic, anti-inflammatory, antibacterial, anticancer, antimalarial. It contains various diterpene lactones; among them, andrographolide occurs in high amounts. Also, it contains flavonoids such as andrographidine, apigenin, and luteolin. Rafi M *et al.*, [47] studied the effect of different concentrations of solvents on the extraction process and monitored its impact on the composition of extracts obtained. In this experiment, extraction was performed using water, 30% ethanol, 50% ethanol, 70% ethanol, and pure ethanol. Extracts were analyzed by HPLC. Overall, 23 peaks in a similar pattern were detected in all extracts. Only the difference in peak height and concentration of extracted components was found with different compositions of extracting solvents. Andrographolide is a main bioactive compound that was determined, and its yield was highest, *i.e.* 114.56 mg/g in 50 % ethanol extract as compared to other solvents. Thus, it is estimated that solvent polarity affects the level of andrographolide extracted. As only from HPLC chromatograms differentiation was not possible, principle component analysis (PCA) was used to classify the extracts according to constituents present. The peak area of a total of 8 major peaks was used as a variable. Samples with a similar profile of metabolites were grouped in one group from a sample with different profiles using PC1 and PC2, two principal components, which explain the difference in analysis. Combining the HPLC fingerprint with PCA classified *Andrographis paniculata* extracts according to solvent extraction. Collection time, geographical region, storage conditions, or post-harvesting conditions has an impact on the quality of plant material; it may lead to changes in the level of phytoconstituents, their yield can hamper.



Name	R_1	R_2	R_3	R_4
Bacooside A_3	[β-D-glucopyranosyl-(1→3)]-o-{α-L-arabinofuranosyl-(1→2)}-o-(β-D-glucopyranosyl]	H		H
Bacopaside II	[α-L-arabinofuranosyl-(1→2)- {β-D-glucopyranosyl-(1→3)}-β-D-glucopyranosyl]	H	H	
Jujubogenin	H	H		H
Bacosaponin C	[β-D-glucopyranosyl-(1→3)]-{α-L-arabinofuranosyl-(1→2)}-α-L-arabinopyranosyl]	H	H	

FIG. 2: COMPOUNDS ANALYZED BY HPLC, HPTLC

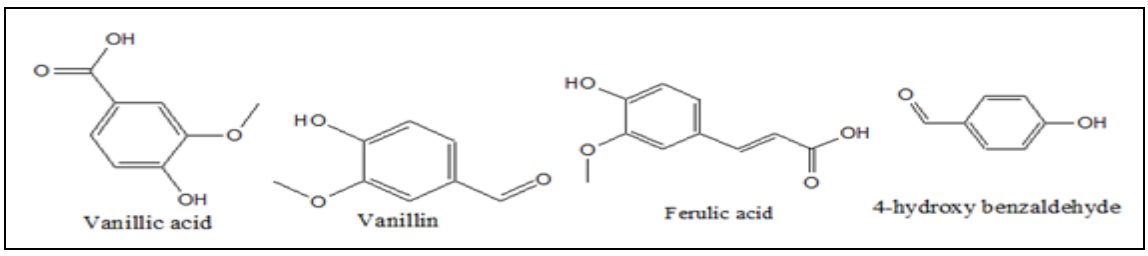


FIG. 3: COMPOUNDS ANALYZED BY ELECTROPHORESIS

A study conducted by Mahanta BP *et al*., [48] on *Curcuma caesia*, commonly known as black turmeric belonging to the family Zingiberaceae, traditionally its rhizomes have been utilized for leprosy, asthma, bronchitis, epilepsy, piles, *etc*. Results of earlier studies on black turmeric by GC-MS were found to have large variations of phyto-constituents present. Hence, the development of an alternative method was a need. In this study, samples collected in summer, monsoon, and post-monsoon season were analyzed. Initially performed GC-MS analysis of crude black turmeric oil turned to be erroneous as inaccurate identification through mass spectrum library-based hit, thermolability phytochemicals. But further, a reliable 1H NMR spectroscopic method was developed using sesquiterpene chemical markers and it was applied for the samples; kept under different storage conditions. From fresh and grounded rhizome essential oil was extracted through hydrodistillation using Clevenger type apparatus and fractionated by silica gel and argenation column chromatography. Fractions were analyzed by TLC and ^{1}H-NMR spectroscopy and pure marker compounds *viz*. furanodiene, furanodienone, curzerenone, germa-crone of the sesquiterpenoid class were isolated. Individual chemical markers and total furano-sesquiterpenoids (FS) were quantified using furfural as an internal standard in deuterated chloroform solvent. Characteristic peaks in ^{1}H-NMR spectra at δ_H 7.07-7.09 (s) ppm for α-furan protons, a key signal at δ_H 1.90-2.20 and δ_C 8.8-9.6 ppm for the presence of β-furan methyl group, two peaks for unsaturation and vinylic methyl each reveal the structure of furanosesquiterpene skeleton (furanodiene, furanodienone, curzereone) whereas four methyl groups in δ_H 1.44-1.77 ppm range for germacrone were identified and quantification was performed based on NMR data. Significant variations in essential oil yield and composition from samples collected in different seasons were observed. Samples collected in monsoon found to be devoid of germacrone when compared to samples collected in summer and post-monsoon. Furanodienone and curzerenone found in a varied amount in the range of 78.8-209.1 mg/g and 319.5-459.8 mg/g. Storage at 4 °C for 3 months lowered down furanodienone and increased curzerenone levels. Air drying and storage at 60 °C showed the same trend. However, storage at room temperature (32±3 °C for 3 months) drastically decreased oil levels and generated complex spectra probably due to degradation.

Therefore this study recommends the use of a fresh or cold-stored sample of black turmeric for essential oil extraction as it gave maximum yield and unaltered composition. The developed NMR method can be applied to the analysis of black turmeric oil and its phyto-formulations.

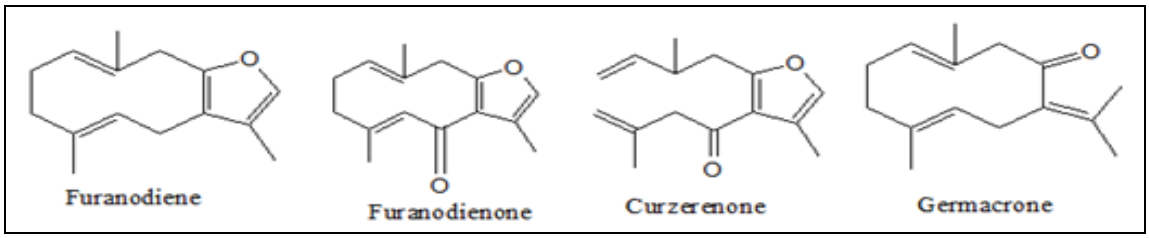


FIG. 4: COMPOUNDS ANALYZED BY NMR

Oroxylum indicum is a traditional Indian herb belonging to family Bignoniaceae, used as a tonic, anti-inflammatory, nootropic, diuretic, *etc*. Also, it is used in ayurvedic formulations like Dasamoola-rishta, Chyavanprash, Amratarista, *etc*. Whereas *Scutellaria baicalensis* is a traditional Chinese herb belonging to family Lamiaceae, its roots are used as anti-inflammatory, anti-viral, for diarrhea, hypertension, *etc*. Majeed *et al*., [49] studied chroma-tographic differentiation of *Oroxylum indicum* and *Scutellaria baicalensis*, both plants are rich in flavonoid content and have major similarities in phytoconstituent content. HPLC, LC-MS, and UV fingerprinting analysis were done to detect chemical similarities and differences based on flavonoid content in both plants. HPLC finger-printing of hydro-alcoholic extracts of *Oroxylum indicum* bark and *Scutellaria baicalensis* roots showed characteristic peaks for flavonoids and further studied by UV and mass fragmentation. By combining this data major compounds identified were baicalin, oroxylin- A- 7- O- glucuronide, chrysin- 7- O- glucuronide, baicalein, chrysin, oroxylin A in both plants in 50 min HPLC run time

and wogonin, skullcap flavone II; additional compounds were found in *Scutellaria baicalensis*. In the HPLC chromatogram, the difference in run time between 44-50 min was observed, where additional two peaks for wogonin (at 45.1 min) and skullcap flavone II (at 47.6 min) were present in *Scutellaria baicalensis*. Developed HPLC, LC-MS methods could well separate and identify all the constituents. It can be applied to quality control of both plant material and their phytoconstituents.

CONCLUSION: As the popularity, the use of herbal products or herbal medicines has increased and its quality determination is of prime importance. With this, events of adulteration or fraudulence are becoming larger in herbal products. The difference between various plant species is important from a quality point of view. Detection of adulteration is needed from various points such as user's safety, maintaining a good position in the market, compliance with rules and regulations of governing bodies. Hence it is necessary to undertake certain approaches for quality evaluation that protects the integrity of herbs, herbal products as a quality medicine for use as 'phytopharmaceutical'. Routine testing methods such as macroscopy, microscopy does not suffice whole quality control, standardization purpose. Chemical evaluation plays a crucial part in standardization. Evaluation of marker compounds and fingerprint analysis are crucial parameters to monitor qualitative and quantitative evaluation. These analyses are performed using different analytical techniques includes spectroscopic and chromatographic methods. Among all techniques, TLC, HPTLC, and HPLC are most commonly used. Whereas spectral analysis by UV and IR are found to be most useful over other tedious methods in certain cases, which fulfill the quality control purpose as compare to chromatographic procedures. The application of these approaches for herbs, herbal products, and herbal medicines quality assessment was presented. A combination of spectral techniques with advanced statistical tools resolves the problems in handling the large data of the number of samples. Considering an enormous number of articles in this context, this article was limited to a narrow field of application in adulteration, quality control, and standardization of herbs, herbal products. We can conclude that analytical tools are being employed for phytochemical standardization.

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REFERENCES:

1. Hexa Research, Herbal medicine market size to reach USD 117.02 Billion By 2024 [Internet] 2017 [updated 2020; cited 2020 Apr 3]. Available from: https://www.hexaresearch.com/press-release/herbal-medicine-market
2. Srivastava S and Misra A: Quality Control of Herbal Drugs: Advancements and Challenges. In: Singh B., Peter K V, editors. New Age Herbals [Internet]. Singapore: Springer; 2018 [cited 2020 Apr 3]. Available from: https://link.springer.com/chapter/10.1007/978-981-10-8291-7_10
3. Singh A, Kalaivani M, Chaudhary P, Srivastava S, Goyal RK and Gupta SK: Opportunities and Challenges in Development of Phytopharmaceutical Drug in India-A SWOT Analysis . Journal of Young Pharmacists 2019; 11(3): 322.
4. Vyas S, Kothari SL and Kachhwaha S: Nootropic medicinal plants: therapeutic alternatives for alzheimer's disease. Journal of Herbal Medicine 2019; 100291: 17-18.
5. Koparde AA, Doijad RC and Magdum CS: Natural Products in Drug Discovery. In: ShaguftaPerveen and Areej Al-Taweel, editors. Pharmacognosy - Medicinal Plants. [Internet]. Croatia: Intech Open; 2019 [cited 2020 Apr 5]. Chapter 14. Available from: https://www.intechopen.com/books/pharmacognosy-medicinal-plants/natural-products-in-drug-discovery 10.5772/intechopen.82860.
6. Prakash J, Srivastava S, Ray RS, Singh N, Rajpali R and Singh GN: Current Status of Herbal Drug Standards in the Indian Pharmacopoeia. Phytotherapy Research 2017; 31(12): 1817-23.
7. Indian Pharmacopoeia. Vol 3. Ghaziabad: Indian Pharmacopoeial Commission, Ministry of Health & Family Welfare Govt. of India; 2018.
8. Pharmacopoeia Commissionfor Indian Medicine & Homoeopathy, Ministry of AYUSH, Government of India. [Internet]. Ghaziabad: Pharmacopoeia Commission for Indian Medicine and Homoeopathy; 2019. Ayurveda Pharmacopoeial Publication; 2018 [cited 2020 Jun 7]; [about 2 screens]. Available from: https://pcimh.gov.in/show_content.php?lang=1&level=1&ls_id=56&lid=54
9. Indian Council of Medical Research, Medicinal Plant Division [Internet]. New Delhi: ICMR, 2019 [cited 2020 Jun 7]. Available from: http://mpd.icmr.org.in/about_us.php
10. Bhatt A: Phytopharmaceuticals: A new drug class regulated in India. Perspectives in Clinical Research. 2016; 7: 59-61.
11. Osman AG, Haider S, Chittiboyina AG and Khan IA: Utility of alkaloids as chemical and biomarkers for quality, efficacy, and safety assessment of botanical ingredients. Phytomedicine 2019; 54: 347-56.

12. Reflection paper on markers used for quantitative and qualitative analysis of herbal medicinal products and traditional herbal medicinal products; EMEA/HMPC/ 253629/2007; European Medicines Agency, Amsterdam, The Netherlands 2008; 1-6.
13. Guideline on quality of herbal medicinal products /traditional herbal medicinal products DRAFT Revision 3 European Medicines Agency, Amsterdam, The Netherlands 2018; 1-6.
14. Li Y, Shen Y, Yao CL, Guo DA. Quality assessment of herbal medicines based on chemical fingerprints combined with chemometrics approach: A review. Journal of pharmaceutical and biomedical analysis 2020; 185: 113215.
15. Saraf A, Saraf A and Chaturvedi A: Phytochemical analysis and chemical fingerprinting of seeds of *Abrus precatorius* L. Chemical Science Transactions 2018; 7(1): 63-70.
16. Vadivel V, Anand P, Manijkumar S, Rajalakshmi P and Brindha P: Chemical Fingerprints of an India Traditional Herbal Drug Talisapatra (*Abies webbiana*) and Comparison with English yew (*Taxus baccata*). International Journal of Pharmcognosy and Phyto-chemistry Research. 2018; 10(2): 84-91.
17. Kucharska-Ambrożej K and Karpinska J: The application of spectroscopic techniques in combination with chemometrics for detection adulteration of some herbs and spices. Microchemical Journal 2020; 153: 104278.
18. Sharma YR: Elementary of Organic Spectroscopy. S Chand & Company Pvt Ltd, New Delhi, Edition 5, 2013: 75, 96.
19. Maji JK: Near-infrared spectroscopy: a potential new mean of assessing multicomponent polyherbal formulation on way before and after extraction. International Journal of Pharmacy and Pharmaceutical Sciences 2017; 9(5): 121-29.
20. Rohman A, Windarsih A, Hossain MA, Johan MR, Ali ME and Ahmad N: Application of near-and mid-infrared spectroscopy combined with chemometrics for discrimination and authentication of herbal products: A review. Journal of Applied Pharmaceutical Science 2019; 9(3): 137-47.
21. Zhu Y, Chen X, Wang S, Liang S and Chen C: Simultaneous measurement of contents of liquirtin and glycyrrhizic acid in liquorice based on near infrared spectroscopy. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy 2018; 196: 209-14.
22. Kamble B, Athawale N, Gugale A, Advankar A, Ghagare A, Katekhaye S, Kulkarni A and Kanukuntla P: Herbal Drug Development: Challenges and Opportunities. In: Goyal MR, Chauhan DN, editors. Plant-and Marine-based Phytochemicals for Human Health: Attributes, Potential, and Use [internet]. New York: Apple Academic Press Inc; 2018 [cited 2020 May 6] Chapter 1. Available from: https://www.taylorfrancis.com/books/e/9781351251983
23. Kumar D and Sharma U: High-performance thin-layer chromatography: An economical alternative for the quality control of medicinal plants and derived products. Separation Science Plus 2018; 1(2): 100-34.
24. Chawla G and Ranjan C: Principle, instrumentation, and applications of UPLC: a novel technique of liquid chromatography. Open Chemistry Journal 2016; 3: 1-16.
25. Wu T, Yu C, Li R and Li J: Minireview: Recent advances in the determination of flavonoids by capillary electrophoresis. Instrumentation Science & Technology 2018; 46(4): 364-86.
26. Rahman M: Application of computational methods in isolation of plant secondary metabolites. In: Sarkar S D, Nahar L, editors. Computational Phytochemistry. [Internet]. Elsevier; 2018 [cited 2020 May 4]. Chapter 4. Available from: https://www.sciencedirect.com/science/ article/pii/B9780128123645000043?via%3Dihub
27. Ganzera M and Sturm S: Recent advances on HPLC/MS in medicinal plant analysis - An update covering 2011- 2016. Journal of pharmaceutical and biomedical analysis. 2018; 147: 211-33.
28. Quirós-Guerrero L, Albertazzi F, Araya-Valverde E, Romero RM, Villalobos H, Poveda L, Chavarría M and Tamayo-Castillo G: Phenolic variation among *Chamae cristanictitans* subspecies and varieties revealed through UPLC-ESI (-)-MS/MS chemical fingerprinting. Metabolomics 2019; 15(2): 14.
29. Oza MJ and Kulkarni YA: A novel contribution to quality standards of *Trifolium pretense* L., A dietary supplement. Indian Journal of Natural Products and Resources 2019; 9(4): 311-30.
30. Choudhary S, Kaur IP and Malik J: Development and validation of a novel, rapid gradient HPLC method for simultaneous estimation of bioactive marker compounds in a mixture of *Convolvulus pluricaulis*, *Withania somnifera* and *Bacopa monnieri* Extracts. Journal of Chromato-graphic Science 2019; 57(10): 920-30.
31. Shaikh S and Jain V: A novel reverse-phase high-performance liquid chromatographic method for simultaneous estimation of ellagic acid, quercetin, and piperine in ayurvedic formulations. Asian Journal of Pharmaceutical and Clinical Research 2018; 11(6): 312-17.
32. Ye Zi, Dai JR, Zhang CG, Lu Y, Wu LL, Gong AG, Xu H, Tsim KW and Wang ZT: Chemical differentiation of *Dendrobium officinale* and *Dendrobium devonianum* by using HPLC fingerprints, HPLC-ESI-MS, and HPTLC analyses. Evidence-Based Complementary and Alternative Medicine 2017; 8647212: 1-9.
33. Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E and Cañigueral S: Comprehensive HPTLC finger-printing as a tool for a simplified analysis of purity of ginkgo products. Journal of Ethnopharmacology 2019; 243: 112084.
34. Upadhye AS, Rajopadhye A and Dias L: Development and validation of HPTLC fingerprints of three species of Alpinia with biomarker Galangin. BMC Complementary and Alternative Medicine 2018; 18(1): 16.
35. Katragunta K, Siva B, Kondepudi N, Vadaparthi PR, Rao NR, Tiwari AK and Babu KS: Estimation of boswellic acids in herbal formulations containing *Boswellia serrata* extract and comprehensive characterization of secondary metabolites using UPLC-Q-Tof-MvadivelSe. Journal of Pharmaceutical Analysis 2019; 9(6): 414-22.
36. Dastjerdi AG, Akhgari M, Kamali A and Mousavi Z: Principal component analysis of synthetic adulterants in herbal supplements advertised as weight loss drugs. Complementary Therapies in Clinical Practice 2018; 31: 236-41.
37. Rocha T, Amaral JS and Oliveira MB: Adulteration of dietary supplements by the illegal addition of synthetic drugs: a review. Comprehensive Reviews in Food Science and Food Safety 2016; 15(1): 43-62.
38. Khare B, Mishra MK, Kesharwani L. Screening of Adulterants in Slimming Herbal Formulation by FTIR Analysis. European Journal of Medicinal Plants. 2019; 30(2):1-8.

39. Ordoudi SA, Cagliani LR, Melidou D, Tsimidou MZ and Consonni R: Uncovering a challenging case of adulterated commercial saffron. Food Control 2017; 81: 147-55.
40. Yasmin J, Ahmed MR, Lohumi S, Wakholi C, Lee H, Mo C and Cho BK: Rapid authentication measurement of cinnamon powder using FT-NIR and FT-IR spectroscopic techniques. Quality Assurance and Safety of Crops & Foods 2019; 11(3): 257-67.
41. Sharma P, Sharma S, Yadav S, Srivastava A, Varma A, Luhana K and Shrivastava N: Categorization of *Saraca asoca* and *Polyalthia longifolia*, using chemical and genetic fingerprinting associated with multivariate statistical analysis. Journal of Applied Research on Medicinal and Aromatic Plants 2018; 9: 91-6.
42. Vadivel V and Brindha P: Phytochemical investigation for the authentication of Indian traditional herbal drug *Boerhavia diffusa* and its differentiation from morpho-logically similar plant *B. erecta*. International Journal of Pharmaceutical Chemistry 2017; 7(6): 86-94.
43. Anubala S, Sekar R and Nagaiah K: Determination of curcuminoids and their degradation products in turmeric (*Curcuma longa*) rhizome herbal products by non-aqueous capillary electrophoresis with photodiode array detection. Food Analytical Methods 2016; 9(9): 2567-78.
44. Kulloli RN: Regional level threat assessment of *Commiphora wightii* (Arn.) Bhandari (Burseraceae) in India. Vegetos An International Journal of Plant Research & Biotechnology 2018; 31(3): 62-67.
45. Ahmed R, Wang YH, Ali Z, Smillie TJ and Khan IA: HPLC method for chemical fingerprinting of Guggul (*Commiphora wightii*)-quantification of E-and Z-guggulsterones and detection of possible adulterants. Plantamedica 2016; 82(4): 356-61.
46. Gomes AF, Ganzera M, Schwaiger S, Stuppner H, Halabalaki M, Almeida MP, Leite MF, Amaral JG and David JM: Simultaneous determination of iridoids, phenylpropanoids and flavonoids in *Lippia alba* extracts by micellar electrokinetic capillary chromatography. Microchemical Journal 2018; 138: 494-00.
47. Rafi M, Devi AF, Syafitri UD, Heryanto R, Suparto IH, Amran MB, Rohman A, Prajogo B and Lim LW: Classification of *Andrographis paniculata* extracts by solvent extraction using HPLC fingerprint and chemometric analysis. BMC Res Notes 2020; 13(1): 1-6.
48. Mahanta BP, Sut D, Kemprai P, Paw M, Lal M and Haldar S: A 1H-NMR spectroscopic method for the analysis of thermolabile chemical markers from the essential oil of black turmeric (*Curcuma caesia*) rhizome: application in post-harvest analysis. Phytochemical Analysis 2020; 31(1): 28-36.
49. Majeed M, Pande A, Nagabhushanam K, Majeed S and Pande A: Comparative chemical fingerprinting of *Oroxylum indicum* and *Scutellaria baicalensis* using liquid chromatography and mass spectrometry. Journal of Liquid Chromatography & Related Technologies 2017; 40(14): 732-40.

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