An overview of the Importance of Quality Evaluation and Validation of Medicinal Plants

Shibu Narayan Jana[1], Anindya Sundar Datta[1], Kousik Santra[1], Sk Mosiur Rahman[1], Md. Harun Al Rashid[2]

[1]Department of Pharmacy, Anand College of Pharmacy, Debra, West Bengal, India, [2]Department of Pharmacy, Samsi RH, Malda, West Bengal, India

Abstract

Phytoconstituents of medicinal plants and their derived formulations are evaluated for quality, safety, and efficacy. In recent years, most people are turning to the use of natural compounds or herbs or their formulations for the prevention or management of different disease conditions. Since phytotherapy is a common component of medical treatment, its efficacy and safety should be required, and the necessity of pharmacological, toxicological, and clinical trials is evident, as noted by Bauer and Tittel nearly 20 years ago. Different methods, such as physical, chemical, and biological, are used for the quality evaluation and standardization of herbal products and their formulations. For metabolomics profiling, a range of chromatographic and hyphenation with spectroscopic techniques, such as high-performance thin layer chromatography, high-performance liquid chromatography, liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, near infrareds, high-performance thin layer chromatography–mass spectrometry, and high-performance liquid chromatography–mass spectrometry, are the effective analytical approaches for quality measurement and standardization of medicinal plants and their extracts and herbal formulations. The processes of a quality evaluation and standardization paradigm are described in this article with the goal of ensuring and preserving the clinical efficacy, quality and safety, and consistent biologically active molecules of medicinal plants and their products.

Key words: Gas chromatography–mass spectrometry, high-performance thin layer chromatography, high-performance liquid chromatography, liquid chromatography–mass spectrometry, medicinal plants, near infrared, quality evaluation, standardization

INTRODUCTION

Medicinal products and their formulations have been used for centuries to prevent and manage different diseases worldwide, also providing nutritional benefits. Medicinal plants and their derived products offer a rich source of biologically active metabolites with effective therapeutic potential. According to the World Health Organization (WHO), 80% of the world population depends on medicinal plants and their formulations.[1] There is a great demand for herbs and medicinal plants worldwide, due to herbs and herbal products having great therapeutic potential in the world market.[2,3] Medicinal plants are used in various forms, i.e., leaves, flowers, fruits, woods, barks, roots, gums, resins, essential oils, etc., and formulations such as extracts, tinctures, etc., for the prevention and management of diseases.[4] In India, AYUSH systems of medicine are used for preventing and managing diseases.[5-7] Over the past few decades, less toxicity, interest of the public as well as acceptance of herbal compounds and their extracts has increased worldwide.[8] One or more extracts or herbs are recommended for synergistic action. No single herb is recommended for a particular health condition.[9] Phytomarkers and biologically active molecules in medicinal Plants responsible for their pharmacological properties.[10] These moieties depend on factors such as plant species, harvesting time, and nature of the soil.[11] In most

cases, the active ingredients behind the biological activities of herbs are not certain, and their therapeutic activity is lower as comparatively to synthetic medicines. These drugs are used very effectively against chronic diseases with a low incidence of side effects.[12] Phytmolecules are used in a variety of field of studies, such as validation of original species, exploration for novel products and materials, extraction and purification process optimization, structural elucidation, and quality evaluation.[13] Phytomarkers through scientific investigation can contribute to the identification and discovery of novel medications.[14,15] The present study aims to provide an overview of the significance, methods, and factors involved in guaranteeing the efficacy, safety, and quality of medicinal plants and their products. A few important medicinal plants, their marker compounds, analytical methods, and pharmacological activities are shown in Table 1.

QUALITY CONTROL AND VALIDATION OF MEDICINAL PLANTS AND THEIR PRODUCTS

It is a systematic approach that involves surveillance, monitoring, and controlling different aspects of medicinal plants and their products development, manufacturing, and distribution to maintain product quality and importance to product safety, efficacy and acceptance. These are very essential process in the pharmaceutical industry.[16,17] Essentially, quality control depends on identity, purity, and content of active botanicals.[18] Extraction type, extraction solvent polarity, and component instability also affect herb quality.[19] Regulation of norms of the medicinal products is lenient when compared to the synthetic medicines. Because of this, quality of the herbal products is decreasing due to false medication.[20] It can lead to adverse effects on the health of the consumers. Hence, controlling the quality of the herbal products and their preparations is very much required for the betterment of humankind.[14]

Table 1: Important medicinal plants, major marker compounds, analytical method, and their pharmacological activity

Medicinal plant	Marker compounds	Analytical method	Pharmacological activity	References
Andrographis paniculata	Andrographolide, Neoandrographolide, Isoandrographolide, Apigenin	HPLC, HPTLC, HPTLC-MS, NIRs	Treatment in fever, inflammation, upper respiratory tract infection	[38,39]
Allium sativum	Ajoenes, allicin, and alliin	HPLC, HPTLC/UPLC-MS	Hypoglycemic, hypolipidemic, antihypertensive	[40]
Aloe vera	Emodin, P-coumaric, gallic, vanilic, ferulic, cinamic and ellagic acids.	HPLC, UV-Vis, HPTLC	Wound healing, anti-inflammatory, hypoglycemic	[41,42]
Azadirachta indica	Nimbanene, nimbidin, and nimbin,	HPTLC, GC-MS	Antibacterial, antimalarial	[43]
Berberis vulgaris	Berlambine, hydroxycanthine, tejedine	LC MS/MS	Used to treat skin infections	[44]
Bacopa monnieri	Bacoside, apigenin, brahmine	HPLC	Memory improver, anti-influenzal	[45,46]
Boswellia serrate	Boswellic acid, lupeolic acid, and acetyl lupeolic acid	UPLC-Q-Tof-MS	Used to treat inflammatory bowel disease, rheumatoid arthritis	[47]
Capsicum annum	Capsaicin, caffeic acid, luteolin, and vitexin	GC-MS, FT-IR	Hypocholesterolemic, antiviral, and antibacterial	[48]
Centella asiatica	Asiaticoside, asiatic acid, and centellicin	HPLC	Antimicrobial and antiinfluezal	[49]
Calendula officinalis	α-cadinol, cadinenes, and copane	HPTLC	Used in treatment of wounds	[50]
Curcuma longa	Curcumin, cyclocurmin, coumaric, and sinapic acid	HPTLC, NIRs	Anti-tumor, antioxidant, anti-arthritic	[51,52]
Citrus limon	Sabinene, 3-carene, limonene	LC-MS/MS	Used for coughs and sore	[53]
Digitalis lanata	Digitoxin, digitoxigenin, and lanatoside C	RP-TLC	Digoxin is used as an antiarrhythmic and inotrope	[54]
Emblica officinalis	Gallic acid, methyl gallate, ellagic acid	HPLC	Antioxidant, anti-inflammatory, adaptogenic	[55]
Ephedra sinica	Ephedrine, pseudoephedrine, N-methylephedrine	LC–MS/MS	Bronchodilators and decongestants	[56]

(Contd...)

Medicinal plant	Marker compounds	Analytical method	Pharmacological activity	References
Erythroxylum coca	Cocaine	GC-MS	Anesthetic	[57]
Ferula assa-foetida	Carvacrol, α-bisabolol, β-ocimene	GC-MS	Hypocholesterolemic and antiasthmatic	[58,59]
Fumaria officinalis	Chelidonine, protopine, and stylopine	MS and MS/MS	Used in skin infections and conjunctivitis	[60]
Gingko biloba	Ginkgolide A, ginkgolide B, and ginkgoside B	LC-HRMS	Antioxidant, anti-Alzheimer	[61]
Glycyrrhiza glabra	Glycyrrhizin, glabridin, glycyrrhizin, glabridin licochalcone A	RP-HPTLC	Anti-inflammatory, antiulcer, antioxidant, hepatoprotective, antibacterial, and antiviral	[62,63]
Geranium robertianum	Ellagitannin, hexahydroxydiphenylglucose	HPLC	Used in wound healing, tooth decay	[64]
Hypericum perforatum	Hypericin, chlorogenic acid	HPLC-ESI-Q-TOF MS	Antidepressant	[65]
Lavandula angustifolia	Camphene, sabinene	SPME-GC-MS	Antiseptic and antipsychotic	[66]
Lawsonia inermis	Eugenol, hexadecanoic acid, phytol, and α-terpineol	TLC	Antibacterial, analgesic and anti-inflammatory	[67]
Momordica charantia	Momordicine I, momordicine II,	PYR-GC/MS	Hypoglycemic and adaptogenic	[68]
Morinda citrifolia	Americanin A, asperuloside, citrifolinin B	HPLC	Used for arthritis, joint pain, and skin infections	[69]
Nelumbo nucifera	Betulinic acid, nelumnucifoside A and nelumnucifoside B	LC-MS/MS	Anti-influenzal, anti-inflammation, anticancer	[71]
Ocimum sanctum	Carvacrol	LC-MS	Oxidative stress, microbial infections, and hypertension	[72]
Piper nigrum	Piperine	HPTLC, Raman spectroscopy	Antiageing, adaptogenic, and immunomodulator, antidiabetic	[72-74]
Piper methysticum	Kavain, methysticin, yangonin	UPLC-MS/MS	Antiasthamatic	[75]
Silybum marianum	Silibinin, silychristin, isosilybin a, taxifolin, Silandrin	UHPLC-MS/MS	Hepatoprotective	[76]
Terminalia belerica	Gallic acid	LC-MS	Anti-atherosclerotic, hepatoprotective, cardioprotective	[77]
Terminalia chebula	Punicalagin, terflavin A, terchebulin, terchebin, and neo-chebulic acid	LC, UPLC-MS/MS	Liver protective, cardiotonic, anticancer and antimicrobial	[78]
Withania somnifera	Withanolide A, withanolide B, withanone, withasomnine, withanoside iv	HPLC	Anti-tumor, antituberculosis, and antiulcer	[79]
Youngia japonica	Chlorogenic acid, chicoric acid, caftaric acid	Light and scanning electron microscopy	Antitussive, used in the treatment of boils and snakebites	[80]
Zingiber officinale	Gingerol, β-bisabolene	HPTLC	Antiviral, antimicrobial	[81]

HPTLC: High-performance thin-layer chromatography, HPLC: High-performance liquid chromatography, LC-MS: Liquid chromatography–mass spectrometry, GC-MS: Gas chromatography–mass spectrometry, NIRs: Near infrareds, HPTLC-MS: High-performance thin-layer chromatography–mass spectrometry, HPLC-MS: High-performance liquid chromatography–mass spectrometry, UPLC-MS: Ultra-performance liquid chromatography–mass spectrometry, UV-Vis: Ultraviolet visible, UPLC-Q-Tof-MS: Ultra-performance liquid chromatography-quadrupole time-of-flight–mass spectrometry, FT-IR: Fourier-transform infrared, RP-HPTLC-MS: Reversed-phase high-performance thin-layer chromatography, LC-HRMS: Liquid chromatography-high-resolution mass spectrometry, SPME-GC-MS: Solid-phase microextraction-gas chromatography–mass spectrometry, PRY-GC-MS: Pyrolysis-gas chromatography–mass spectrometry, HPLC-ESI-Q-TOF: High-performance liquid chromatography-electrospray ionization-quadrupole-time-of-flight, RP-TLC: Reverse phase thin-layer chromatography, UPLC-MS/MS: Ultra-performance liquid chromatography–tandem mass spectrometry, UHPLC-MS/MS: Ultra-high-performance liquid chromatography–tandem mass spectrometry
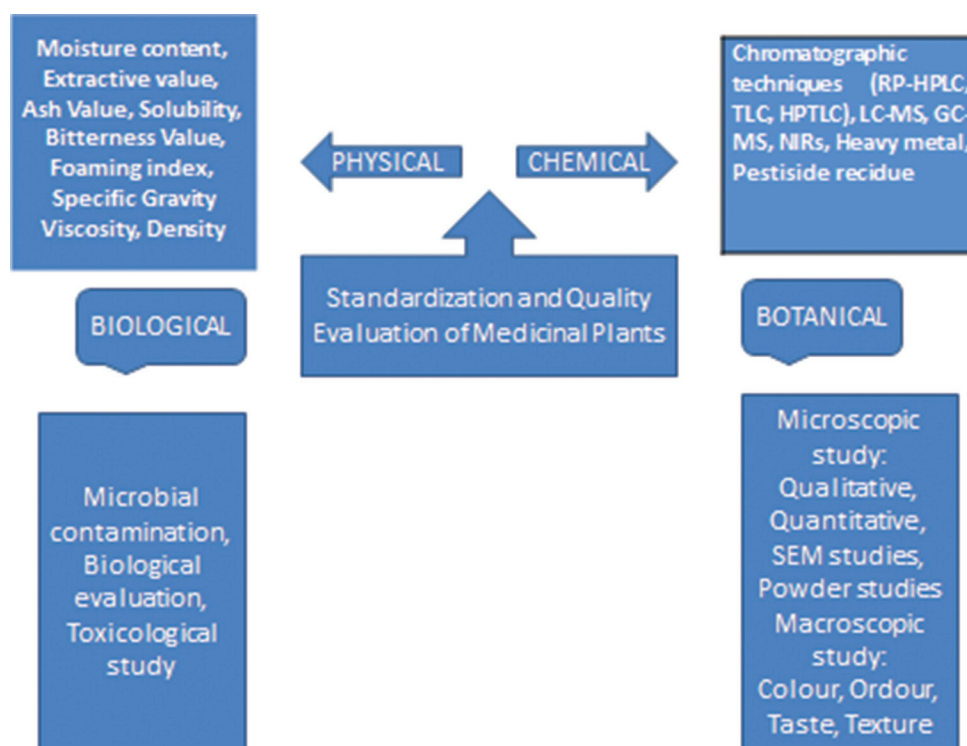
Figure 1: Flowchart of standardization and quality evaluation techniques of medicinal plants

Medicinal plant	Mobile phase	Spraying reagent	Visible region	Marker Compound	References
Clitoria ternatea L	Hexane: Ethyl acetate (4:1 v/v).	Anisaldehyde reagent	420 nm	Taraxerol	[82]
Vitex trifolia	Chloroform: Acetone (98:2, v/v)	Vanillin/sulfuric acid reagent	610 nm	Diterpenoids	[83]
Ruellia tuberosa	Chloroform: Toluene: Ethyl acetate (6: 3: 1, v/v)	Anisaldehyde sulfuric acid reagent	366 nm	Retension factor (Rf) Value 0.56 was selected as marker compound	[84]
Terminalia sericea	Dichloromethane: ethyl acetate: Methanol: Formic acid (90:10:30:1)	10% Sulfuric acid in methanol	White light	Resveratrol-3-O-β-rutinoside	[85]
Myrica esculenta	Toluene: Ethyl acetate: Formic acid: Methanol (3:3: 0.6: 0.4)	5% Sulfuric acid in methanol	366 nm	Myricetin	[86]
Holostemma ada-kodien	Toluene: Ethyl acetate: Formic acid (5: 3: 0.1)	Vanillin-sulfuric acid reagent,	Visible light	The methanol extract under visible light in track I showed a maximum area of 46.87%, spanning from Rf 0.73 to 0.98, and in track II, it displayed a maximum area of 36.59%	[87]
Fumaria parviflora	Chloroform: Ethylacetate: Formic acid (5:4:1).		254 nm and 366 nm	At 254 nm, it represents 11 and 10 peaks for track 1 and track 2, respectively, whereas at 366 nm, 9 and 12 peaks for track 1 and track 2, respectively.	[88]

Table 2: Quality evaluation of some Indian medicinal plants using HPTLC

HPTLC: High-performance thin-layer chromatography

Table 3: Quality control of some important medicinal plants using LC-MS studies

Medicinal plant	Phyto-marker	Biological activity	References
Valeriana jatamansi	Valerenic acid	antitumour	[89]
Michelia champaca	Phlorizin and astilbin	Hypoglycemic	[90]
Zingiber officinale	Pyrogallol, p-hydroxybenzoic acid, ferulic acid and p-coumaric acid	Antioxidant	[91]
Mangifera indica	Neomangiferin, mangiferin, kaempferol-3-O-rutinoside, isoquercetin and quercetin	Antioxidant, hypoglycemic	[92]
Salvia miltiorrhiza	Cryptotanshinone, trijuganone B, and 15,16-dihydrotanshinone I	Used for the treatment of cardio-cerebrovascular diseases, cirrhosis, cancer, and osteoporosis	[93]
Hedyotis aspera	14,19-Dihydroaspi dospermatine, coumeroic acid, lycocernuine and muzanzagenin	Nephroprotective	[94]
Andrographis paniculata	Neoandrographolide, andrographolactone, 14-dehydroxy-11,12-didehydroandrographolide, skullcapflavone I, and 5-Hydroxy-2′,7,8-tri methoxy flavone	Anti-malarial	[95]
Euphorbia hirta	Neochlorogenic acid, quercetin-3β-D-glucoside, syringic acid, caffeic acid, ellagic acid, astragalin, afzelin, quercetin, corchorifatty acid F, 2-undecanone 2,4-dinitrophenylhydrazone, and demexiptiline.	Anti-gout	[96]

LC-MS: Liquid chromatography–mass spectrometry

Table 4: Quality evaluation of some Indian medicinal plants using FT-IR method

Medicinal plant	FT-IR analysis	References
Polyherbal formulation: a. *Dillenia indica* b. *Trigonella foenum graecum* c. *Cuminum cyminum*	FT-IR analysis of chloroform, water, and ethyl acetate extracts revealed several functional groups. Alkenes (C=C bending at 721 cm^{-1}) were present in all extracts. Amines/Fluoro compounds/Tertiary alcohols/Alkyl aryl ethers (C-N stretching at 1161 cm^{-1} and C-F stretching at 1231 cm^{-1}) were also found in all extracts. C=O stretching (1686 and 1746 cm^{-1}) was observed in chloroform and ethyl acetate extracts. The chloroform extract also showed O-H bending and C-F stretching at 1377 cm^{-1}. Importantly, 11 bands across all extracts indicated the presence of alcohols (O-H stretching)	[97]
Momordica balsamina	FT-IR analysis revealed six major peaks, confirming the presence of alkaloids, flavonoids, terpenes, anthraquinones, and phenolic compounds. A peak at 3384.84 cm^{-1} suggested O-H (phenols, alcohols) or N-H (amines, amides) stretching, likely indicating phenolic compounds and possibly alkaloids. A peak at 2938.72 cm^{-1} (C-H stretching) pointed to terpenes. A peak at 2039.40 cm^{-1} (C≡C stretching) suggested alkynes. A peak at 1637.18 cm^{-1} was in a complex region, potentially indicating aromatic rings, amino acids, flavonoids, or C=C stretching (alkenes). Finally, peaks at 1084.85 and 1029.61 cm^{-1} (C-O stretching) suggested esters or secondary alcohols.	[98]
Operculina terpathum	Peaks at 3893.01 and 3860.20 cm^{-1} suggested organic acids (-COOH). Hydroxyl and phenolic compounds were indicated by peaks at 3522.6 and 3421.74 cm^{-1}. Aliphatic compounds (C–H bend) and esters were identified by peaks at 3138 and 1632.63 cm^{-1}, respectively. Peaks at 1343.42, 1148.16, and 1074.91 cm^{-1} suggested amides, ketones, aldehydes, aromatics (C–C and C-O stretch), and aliphatic amines (C–N stretch). Finally, peaks from 988.74 to 608.56 cm^{-1} indicated halogen compounds (C-Cl, C-F, C-Br).	[99]
Combretum album	FT-IR analysis of an ethanolic leaf extract showed major bands at 2923.56, 1608.34, 1282.43, 1175.4, and 1014.37 cm^{-1}. These bands suggest the presence of alcohols (3400–3200 and 1075–1000 cm^{-1}), aromatics (1040–995 and 730–680 cm^{-1}), amines (1146–1132 and 3500–3300 cm^{-1}).	[100]

FT-IR: Fourier-transform infrared

Table 5: Quality evaluation of some Indian medicinal plants using GC-MS method

Medicinal plant	Biological activity	Phyto-marker	References
Rhazya stricta	Used in the treatment female reproduction diseases, cardiovascular diseases, obesity, inflammatory conditions, and liver disorders	methyl stearate, Methyl palmitate, methyl tetradecanoate	[101]
Amomum nilgiricum	Antibacterial and antifungal	Serverogenin acetate	[102]
Citrullus colocynthis	Strong antimicrobial	Isooctylphthalate	[103]
Hibiscus asper	Antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer	9,12,15-octadecatrien-1-ol, n-Hexadecanoic acid, octadecatrienol acid, methyl palmitate, and phytol.	[104]
Amomum tsaoko	Gastrointestinal regulation, blood sugar reduction properties and antibacterial	Copaene, 2-octylfuran, 2,3-dihydro-1H-indene-4-carbaldehyde	[105]
Tylophora pauciflora	n-Hexadecanoic acid has the highest peak area and it Its properties include anti-oxidant, 5-alpha-reductase-inhibitor, anti-fibrinolytic, hemolytic, antibacterial activity, hypocholestemic nematicide, pesticide	The n-hexadecanoic acid, Octadecadienoic acid, Nonenoic acid, methyl ester	[106]
Papaver decaisnei	Antiradical, and antitumor	Decarbomethoxy- tabersonine, hexadecanoic acid, and anthocyanin. In the leaves, the major alkaloids identified in the methanol extract were 9,12,15-octadecatrien-1-ol, hexadecanoic acid, and γ-sitosterol.	[107]

GC-MS: Gas chromatography–mass spectrometry

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) METHOD FOR THE QUALITY EVALUATION AND VALIDATION OF MEDICINAL PLANTS

It is an excellent and convenient approach for the standardization and validation of herbs and herbal compounds. Applications of HPTLC include phytochemical screening, quantification, fingerprinting, and estimation of adulterants.[21] One important quality control method for determining the pharmacopeial standards of different herbal, Siddha, and ayurvedic medications is the HPTLC fingerprinting instrument. The HPTLC method can be used as a quality control method for identification and is employed concurrently to compare the chemicals in the samples and the reference. The HPTLC fingerprint images provide both qualitative and quantitative data when compared to reference standards. This technique can be applied to determine the minimum content, purity percentage, and marker compound identification. Some important medicinal plants, and their quality evaluation using HPTLC method have been shown in Table 2.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYTICAL METHOD FOR THE QUALITY CONTROL OF BOTANICALS

HPLC is a versatile separation method for qualitative as well as quantitative evaluation of targeted phytomolecules in medicinal plants and herbal extracts. The HPLC technique is generally used in authentication, validation, standardization, and quality evaluation based on the marker compounds, which ensure its quality, safety, and efficacy of medicinal products. HPLC is hyphenated with various detection techniques, such as ultraviolet visible, photodiode array detector, mass spectrometry (MS), and nuclear magnetic resonance, to obtain higher sensitivity and detection capabilities of the phytoconstituents for quality control of herbal medicines.[22] The hyphenated chromatographic techniques, along with chemometric analysis, often offer an excellent approach for the evaluation of the quality as well as efficacy of medicinal plants. In the reverse-phase HPLC (RP-HPLC) technique, the chemical nature of the stationary phase, i.e., the column, is relatively non-polar in comparison

to the mobile phase. The column most widely used is the C18 column, which is better known as the octadecylsilane column. The mobile phase is a mixture of two solvents.[23] One of the solvents is an aqueous phase, consisting of water (HPLC grade), whose Potential of hydrogen (pH) has been adjusted with an orthophosphoric acid or glacial acetic acid to a desired PH and another organic solvent, such as methanol or acetonitrile.

LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (LC-MS) ANALYTICAL METHOD FOR THE QUALITY CONTROL AND VALIDATION OF MEDICINAL PLANTS AND THEIR FORMULATIONS

In complicated matrix analysis, where prior treatment for the concentration and purification of the API is required to simplify the procedure and produce better results, the HPLC method has several limitations when used alone without the combination of other methods. Liquid chromatography–mass spectroscopy, a mass spectroscopy-coupled HPLC technology, overcomes this limitation by significantly increasing detection sensitivity.[24] Ion trap LC-MS, quadrupole time-of-flight high-resolution MS, and triple-quadrupole MS are various techniques that can be integrated with HPLC analytical methods for method simplification. The LC-MS technique can be used to identify, quantify, and control the quality of raw plant material extracts and marketed products. Its capabilities include structure elucidation, molecular mass, fragmentation information, retention time, and a wide range of detection and separation of analytical compounds.[25] A few important medicinal plants, quality control using LC-MS studies are shown in Table 3.

GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC-MS) METHOD FOR THE QUALITY CONTROL AND VALIDATION OF MEDICINAL PLANTS

The analytical method known as gas chromatography–mass spectroscopy combines the use of gas chromatography to separate the various components of chemical compound mixtures with the use of mass spectroscopy to analyze the components that the GC is separating. GC-MS can also be used in the pharmaceutical, cosmetic, and food sectors, as well as environmental and forensic applications, to analyze the components of a substance, primarily active medicinal compounds. Qualitative component identification, component separation, and quantification of various volatile and non-volatile substances are all included in a single analysis. It is feasible to analyze many substances at the same time.[26]

NEAR INFRAREDS (NIRS) TECHNIQUE FOR THE QUALITY CONTROL AND VALIDATION OF MEDICINAL PLANTS AND THEIR PRODUCTS

NIR spectroscopy is an attractive analytical tool in medicinal research at the current time, which has been mentioned in the European Pharmacopoeia since 1997.[27] The principles of the NIR method differ from the conventional analytical techniques, such as GC, mass spectroscopy, HPLC, or HPTLC, which are conventionally used in quality evaluation and validation purposes of medicinal plants.[28] This approach is used to estimate quality-related parameters such as starch, amylose, protein, moisture, glycosides, and polyphenolic compounds in medicinal plants. This technique can provide specific, simultaneous, rapid, non-destructive, and accurate estimation of phytochemically active molecules of different medicinal plants and their products. In near-infrared spectroscopy, molecules are excited in the wavelength range of 750–2500 nm. Harmonics and combinations can be found in the NIR range.[29]

STANDARDIZATION OF MEDICINAL PLANTS

Pre-defining the quantity, quality, and therapeutic efficacy of each dose molecule, standardization, and identification of herbs and medicinal plants during the production process and quality control lead to reproducible quality of a specific product.[30] Standardized plant extracts containing specific compounds and quality controlled during the growing, harvesting, and production process. Phyto-marker compound selection basically depends on different factors such as stability, ease of analysis, time and cost of analysis, and therapeutic effect. Controversial identity and adulteration of plant material are very challenging for the standardization of botanicals. Other issues are also related to the standardization of herbal products. The majority of the time, the active components in herbal products are unknown, and reference compounds and specific analytical techniques may not be commercially accessible. Herbal medicines are typically blends of numerous botanicals.[31,32]

MARKER PROFILING

Biolgically active compounds of a medicinal plant or extract that having biologically active properties which are subjected for quality control purposes.[33] Herbal medications must take into account a variety of botanicals for effective quality control, rather than considering one or two individual marker compounds to assess product quality. However, all medicinal plant products and their extracts contain hundreds of unknown ingredients, many in very small amounts.[34,35] It is crucial to get chromatographic fingerprints shown in

Figure 1. Marker profiling by FT-IR) spectroscopy is a rapid, non-destructive analytical method used to produce molecular fingerprint of a phytochemically active molecules. A few important medicinal plants, and their quality evaluation using FT-IR method are shown in Table 4. GC-MS study is used to qualitative component identification, component separation, and quantification of various volatile and non-volatile substances are all included in a single analysis. Some important medicinal plants and their quality evaluation using GC-MS method have been shown in Table 5. Assuring quality control of medicinal plants begins with the identification of the purported plant. In botany, unequivocal nomenclature plays a key role for the precise definition of the vegetal material used in botanical medicines and food supplements, or plants studied by phytochemists. Thus, quality evaluation, validation, chemoprofiling, characterization and standardization are essential tech for determining and quantifying of the active phytomolecule(s) and linking the chemical properties to the pharmacological activities.[36,37]

CONCLUSION

There are numerous factors such as pesticides residue, toxins content, the heavy metals contamination, good agricultural practice, good manufacturing practice, etc. which are affect the quality, safety, and efficacy of medicinal plant materials and its products. Different chromatographic and spectroscopic evaluation leads to increase in the quality and safety of medicinal plants. Standardization is the analytical approach for maintaining the quality, safety, potency and consistency of phytomarkers of herbal products and its formulations. This quality control and standardization techniques will provide required potency, safer and effective use and treatment of herbs and herbal products which will provide a method of well-being for mankind and society.

REFERENCES

1. Mukherjee PK, Nema NK, Venkatesh P, Debnath PK. Changing scenario for promotion and development of Ayurveda-way forward. J Ethnopharmacol 2012;143:424-34.
2. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med 2013;10:210-29.
3. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014;4:177.
4. DeFilipps RA, Krupnick GA. The medicinal plants of Myanmar. PhytoKeys 2018;102:1-341.
5. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol 2006;103:25-35.
6. WHO. WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Vol. 2. set. Vol. 1 Contains Text and Vol. 2. Maps. Geneva: World Health Organization; 2005.
7. Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res 2012;3:200-1.
8. Mukherjee PK, Venkatesh P, Ponnusankar S. Ethnopharmacology and integrative medicine - Let the history tell the future. J Ayurveda Integr Med 2010;1:100-9.
9. Wachtel-Galor S, Benzie IF, editors. Herbal Medicine: An introduction to its history, usage, regulation, current trends, and research needs. In: Herbal Medicine, Biomolecular and Clinical Aspects. 2nd ed., Ch. 1. Boca Raton, FL: CRC Press/Taylor & Francis; 2011.
10. El SMT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, *et al.* Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits - a comprehensive review. Front Immunol 2025
11. Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A. Conservation and sustainable use of medicinal plants: Problems, progress, and prospects. Chin Med 2016;11:37.
12. Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines (Basel) 2015;2:251-86.
13. Kellogg JJ, Paine MF, McCune JS, Oberlies NH, Cech NB. Selection and characterization of botanical natural products for research studies: a NaPDI center recommended approach. Nat Prod Rep 2019;14;36:1196-221.
14. Mukherjee PK, Ponnusankar S, Venkatesh P, Gantait A, Pal BC. Marker profiling: An approach for quality evaluation of Indian medicinal plants. Drug Inform J 2011;45:1-14.
15. Mickymaray S. Efficacy and mechanism of traditional medicinal plants and bioactive compounds against clinically important pathogens. Antibiotics (Basel) 2019;8:257.
16. Mukherjee PK, Houghton PJ. The Worldwide Phenomenon of Increased Use of Herbal Products: Opportunity and Threats, in Evaluation of Herbal Medicinal Products - Perspectives on Quality, Safety and Efficacy. 1st ed. Great Britain: Pharmaceutical Press, Royal Pharmaceutical Society of Great Britain; 2009.
17. Yu LX, Amidon G, Khan MA, Hoag SW, Polli J, Raju GK, *et al*. Understanding pharmaceutical quality by design. AAPS J 2014;16:771-83.
18. Mukherjee PK, Verpoorte R. GMP for Botanicals-Regulatory and Quality Issues on Phytomedicines. 1st ed. New Delhi: Business Horizons; 2003.
19. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chin Med 2018;17:13-20.
20. Sahoo N, Manchikanti P, Dey S. Herbal drugs: Standards and regulation. Fitoterapia 2010;81:462-71.
21. Islam MK, Sostaric T, Lim LY, Hammer K, LocherC.

Development of an HPTLC-based dynamic reference standard for the analysis of complex natural products using Jarrah honey as test sample. PLoS One 2021;16:e0254857.

22. Noviana E, Indrayanto G, Rohman A. Advances in fingerprint analysis for standardization and quality control of herbal medicines. Front Pharmacol 2022;13:853023.

23. Walter TH, Iraneta P, Capparella M. Mechanism of retention loss when C8 and C18 HPLC columns are used with highly aqueous mobile phases. J Chromatogr A 2005;1075:177-83.

24. Pati S, Nie B, Arnold RD, Cummings BS. Extraction, chromatographic and mass spectrometric methods for lipid analysis. Biomed Chromatogr 2016;30:695-709.

25. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. Clin Biochem Rev 2009;30:19-34.

26. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. J Food Sci Technol 2015;52:1212-7.

27. Huck C. Infrared spectroscopic technologies for the quality control of herbal medicines. In: Evidence-Based Validation of Herbal Medicine. Netherlands: Elsevier; 2015. p. 477-93.

28. Beć KB, Grabska J, Huck CW. NIR spectroscopy of natural medicines supported by novel instrumentation and methods for data analysis and interpretation. J Pharm Biomed Anal 2021;193:113686.

29. Huck CW. Recent developments in solid-phase extraction for near and attenuated total reflection infrared spectroscopic analysis. Molecules 2016;21:633.

30. Mukherjee PK. Evaluation of Indian traditional medicine. Drug Inform J 2001;35:631-40.

31. Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, *et al*. Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. Int J Mol Sci 2018;1578.

32. Mukherjee PK, Bahadur S, Harwansh RK, Chaudhary SK. Shifting paradigm for validation of medicinal plants in Indian traditional medicine. Indian Drugs 2014;51:5-14.

33. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacolm 2023;25:1265178.

34. Mukherjee PK. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. 1st ed. India: Business Horizons; 2002.

35. Li S, Tian M, Row KH. Effect of mobile phase additives on the resolution of four bioactive compounds by RP-HPLC. Int J Mol Sci 2010;11:2229-40.

36. Upton R, David B, Gafner S. Botanical ingredient identification and quality assessment: strengths and limitations of analytical techniques. Phytochem Rev 2020;19:1157-77.

37. Ipek S. Importance of ethnopharmacological studies in drug discovery: Role of medicinal plants. Phytochem Rev 2020;19:1199-209.

38. Song YX, Liu SP, Jin Z, Qin JF, Jiang ZY. Qualitative and quantitative analysis of *Andrographis paniculata* by rapid resolution liquid chromatography/time-of-flight mass spectrometry. Molecules 2013;18:12192-207.

39. Low M, Khoo CS, Münch G, Govindaraghavan S, Sucher NJ. An *in vitro* study of anti-inflammatory activity of standardised *Andrographis paniculata* extracts and pure andrographolide. BMC Complement Altern Med 2015;15:18.

40. Oyaluna ZE, Abolaji AO, Bodede O, Olanlokun JO, Prinsloo G, Steenkamp P, *et al*. Chemical analysis of Alliin-Rich *Allium sativum* (Garlic) extract and its safety evaluation in *Drosophila melanogaster*. Toxicol Rep 2024;13:101760.

41. Metcalfe C. Quantitation of *Aloe vera* polysaccharides by O-acetyl and UV-Vis spectrophotometry: First action 2018.14. J AOAC Int 2019;102:1091-4.

42. Hamman JH. Composition and applications of *Aloe vera* leaf gel. Molecules 2008;13:1599-616.

43. Swapna SR, Ramalakshmi K, Udaya SK. Characterization of neem (*Azadirachta indica* A. Juss) seed volatile compounds obtained by supercritical carbon dioxide process. J Food Sci Technol 2018;55:1444-54.

44. Och A, Olech M, Bąk K, Kanak S, Cwener A, Cieśla M, *et al*. Evaluation of the antioxidant and anti-lipoxygenase activity of *Berberis vulgaris* L. Leaves, fruits, and stem and their LC MS/MS polyphenolic profile. Antioxidants (Basel) 2023;12:1467.

45. Kumar N, Abichandani LG, Thawani V, Gharpure KJ, Naidu MU, Venkat Ramana G. Efficacy of standardized extract of *Bacopa monnieri* (Bacognize®) on cognitive functions of medical students: A six-week, randomized placebo-controlled trial. Evid Based Complement Alternat Med 2016;2016:4103423.

46. Dowell A, Davidson G, Ghosh D. Validation of quantitative HPLC method for bacosides in keenmind. Evid based complement Alternat Med 2015;2015:696172.

47. Katragunta K, Siva B, Kondepudi N, Vadaparthi PR, Rama RN. Estimation of boswellic acids in herbal formulations containing *Boswellia serrata* extract and comprehensive characterization of secondary metabolites using UPLC-Q-Tof-MSe. J Pharm Anal 2019;9:414-22.

48. Ciulu-Costinescu F, Neamțu J, Popescu M, Chirigiu L, Simionescu A, Bubulică MV, *et al*. Preliminary analysis of *Capsicum annuum* L. extracts. Curr Health Sci J 2015;41:311-6.

49. da Rocha PB, Souza BD, Andrade LM, Marreto RN, Lima EM, Taveira SF. Development of a high-performance liquid chromatographic method for asiaticoside quantification in different skin layers after topical application of a *Centella asiatica* extract. Planta Med 2017;83:1431-7.

50. Loescher CM, Morton DW, Razic S, Agatonovic-Kustrin S. High performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) for the qualitative and quantitative analysis of *Calendula officinalis*-advantages and limitations. J Pharm Biomed Anal 2014;98:52-9.

51. Kotra VS, Satyabanta L, Goswami TK. A critical review of analytical methods for determination of curcuminoids in turmeric. J Food Sci Technol 2019;56:5153-66.

52. Bagad AS, Joseph JA, Bhaskaran N, Agarwal A. Comparative evaluation of anti-inflammatory activity of curcuminoids, turmerones, and aqueous extract of *Curcuma longa*. Adv Pharmacol Sci 2013;2013:805756.

53. Park YS, Kim ID, Dhungana SK, Park EJ, Park JJ, Kim JH, *et al*. Quality characteristics and antioxidant potential of lemon (*Citrus limon* Burm. f.) seed oil extracted by different methods. Front Nutr 2021;8:644406.

54. Ikeda Y, Fujii Y, Umemura M, Hatakeyama T, Morita M, Yamazaki M. Quantitative determination of cardiac glycosides in *Digitalis lanata* leaves by reversed-phase thin-layer chromatography. J Chromatogr A 1996;746:255-60.

55. Muthuraman A, Sood S, Singla SK. The antiinflammatory potential of phenolic compounds from Emblica officinalis L. in rat. Inflammopharmacology 2011;19:327-34.

56. Sullivan D, Wehrmann J, Schmitz J, Crowley R, Eberhard J. Determination of ephedra alkaloids by liquid chromatography/tandem mass spectrometry. J AOAC Int 2003;86:471-5.

57. Rivier L. Analysis of alkaloids in leaves of cultivated Erythroxylum and characterization of alkaline substances used during coca chewing. J Ethnopharmacol 1981;3:313-35.

58. Niazmand R, Razavizadeh BM. *Ferula asafoetida*: Chemical composition, thermal behavior, antioxidant and antimicrobial activities of leaf and gum hydroalcoholic extracts. J Food Sci Technol 2021;58:2148-59.

59. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin)-a review. J Ethnopharmacol 2011;134:1-10.

60. Sturm S, Strasser EM, Stuppner H. Quantification of *Fumaria officinalis* isoquinoline alkaloids by nonaqueous capillary electrophoresis-electrospray ion trap mass spectrometry. J Chromatogr A 2006;1112:331-8.

61. Cruz MB, Place BJ, Wood LJ, Urbas A, Wasik A, de Carvalho Rocha WF. A nontargeted approach to determine the authenticity of *Ginkgo biloba* L. plant materials and dried leaf extracts by liquid chromatography-high-resolution mass spectrometry (LC-HRMS) and chemometrics. Anal Bioanal Chem 2020;412:6969-82.

62. Alam P, Foudah AI, Zaatout HH, Kamal YT, Abdel-Kader MS. quantification of glycyrrhizin biomarker in *Glycyrrhiza glabra* rhizome and baby herbal formulations by validated RP-HPTLC methods. Afr J Tradit Complement Altern Med 2017;14:198-205.

63. Shi C, Liu J, Yew T. *Ginkgo biloba* extract in Alzheimer's disease: From action mechanisms to medical practice. Int J Mol Sci 2010;11:107-23.

64. Fodorea CS, Vlase L, Suciu S, Tămaş M, Leucuţa SE. Preliminary HPLC study on some polyphenols of *Geranium robertianum* L. (Geraniaceae). Rev Med Chir Soc Med Nat Iasi 2005;109:174-8.

65. Alahmad A, Alghoraibi I, Zein R, Kraft S, Dräger G, Walter JG, *et al*. Identification of major constituents of *Hypericum perforatum* L. extracts in syria by development of a rapid, simple, and reproducible HPLC-ESI-Q-TOF MS analysis and their antioxidant activities. ACS Omega 2022;7:13475-93.

66. Łyczko J, Jałoszyński K, Surma M, Masztalerz K, Szumny A. HS-SPME analysis of true lavender (*Lavandula angustifolia* Mill.) leaves treated by various drying methods. Molecules 2019;24:764.

67. Charoensup R, Duangyod T, Palanuvej C, Ruangrungsi N. Pharmacognostic specifications and lawsone content of *Lawsonia inermis* leaves. Pharmacognosy Res 2017;9:60-4.

68. Brandão DO, Guimarães GP, Santos RL, Júnior FJ, da Silva KM, de Souza FS, *et al*. Model analytical development for physical, chemical, and biological characterization of Momordica *charantia* Vegetable Drug. J Anal Methods Chem 2016;2016:7528297.

69. Westendorf J, Effenberger K, Iznaguen H, Basar S. Toxicological and analytical investigations of noni (*Morinda citrifolia*) fruit juice. J Agric Food Chem 2007;55:529-37.

70. Zhu MZ, Wu W, Jiao LL, Yang PF, Guo MQ. Analysis of flavonoids in lotus (*Nelumbo nucifera*) leaves and their antioxidant activity using macroporous resin chromatography coupled with LC-MS/MS and antioxidant biochemical assays. Molecules 2015;20:10553-65.

71. Chaudhary A, Sharma S, Mittal A, Gupta S, Dua A. Phytochemical and antioxidant profiling of Ocimum sanctum. J Food Sci Technol 2020;57:3852-63.

72. Setyaningsih D, Santoso YA, Hartini YS, Murti YB, Hinrichs WL, Patramurti C. Isocratic high-performance liquid chromatography (HPLC) for simultaneous quantification of curcumin and piperine in a microparticle formulation containing *Curcuma longa* and *Piper nigrum*. Heliyon 2021;7:e06541.

73. Jana SN, Sing D, Banerjee S. Quantification of piperine in different varieties of *Piper nigrum* by a validated high-performance thin-layer chromatography-densitometry method. J Planar Chromat 2021;34:521-30.

74. Sing D, Jana SN, Banerjee S, Mallik R, Majumdar K, Halder PK, *et al*. Rapid estimation of piperine in black pepper: Exploration of Raman spectroscopy. Phytochem Anal 2022;33:204-13.

75. Mamallapalli J, Kanumuri SR, Corral P, Johnston E, Zhuang C, McCurdy CR, *et al*. Characterization of

different forms of kava (*Piper methysticum*) products by UPLC-MS/MS. Planta Med 2022;88:1348-59.

76. Muchiri RN, van Breemen RB. Chemical standardization of milk thistle (Silybum marianum L.) extract using UHPLC-MS/MS and the method of standard addition. J Am Soc Mass Spectrom 2024;35:1726-32.

77. Tiwana G, Cock IE, Cheesman MJ. Phytochemical analysis and antimicrobial activity of *Terminalia bellirica* (Gaertn.) Roxb. and Terminalia chebula retz. fruit extracts against gastrointestinal pathogens: Enhancing antibiotic efficacy. Microorganisms 2024;12:2664.

78. Ou L, Hao Y, Liu H, Zhu Z, Li Q, Chen Q, *et al.* Chebulinic acid isolated from aqueous extracts of Terminalia chebula Retz inhibits *Helicobacter pylori* infection by potential binding to Cag A protein and regulating adhesion. Front Microbiol 2024;15:1416794.

79. Ganzera M, Choudhary MI, Khan IA. Quantitative HPLC analysis of withanolides in Withania somnifera. Fitoterapia 2003;74:68-76.

Bokyung C, Sun YM, Tae SJ, Micromorphological and cytological comparisons between *Youngia japonica* and *Youngia longiflora* using light and scanning electron microscopy. Microscopy Research and Technique.

80. Alam P. Densitometric HPTLC analysis of 8-gingerol in Zingiber officinale extract and ginger-containing dietary supplements, teas and commercial creams. Asian Pac J Trop Biomed 2013;3:634-8.

81. Kumar V, Mukherjee K, Kumar S, Mal M, Mukherjee PK. Validation of HPTLC method for the analysis of taraxerol in *Clitoria ternatea*. Phytochem Anal 2008;19:244-50.

82. Tiwari N, Yadav AK, Gupta MM. Validated HPTLC method for the simultaneous quantification of diterpenoids in *Vitex trifolia* L. J Sep Sci 2013;36:2373-8.

83. Chothani DL, Patel MB, Mishra SH. HPTLC fingerprint profile and isolation of marker compound of *Ruellia tuberosa.* Chromatogr Res Int 2012;2012:180103.

84. Mulaudzi N, Anokwuru CP, Tankeu SY, Combrinck S, Chen W, Vermaak I, *et al.* Phytochemical profiling and quality control of *Terminalia sericea* burch. ex DC. using HPTLC metabolomics. Molecules 2021;26:432.

85. Shah H, Naseer A, Gupta N, Singh R. A study on HPTLC quantification for quality control of myricetin a nutraceutical from different plant parts of *Myrica esculenta* Linn. Biomed Pharmacol J 2022;15.

86. Devipriya D, Radhamany PM. HPTLC fingerprints and FTIR analysis for authentication and quality control of *Holostemma ada-kodien* Schult. Appl Biol Res 2024;26:286-97.

87. Bhargava A, Shrivastava P, Tilwari A. HPTLC analysis of *Fumaria parviflora* (Lam.) methanolic extract of whole plant. Future J Pharm Sci 2021;7:1-9.

88. Shukla V, Singh P, Kumar D, Konwar R, Singh B, Kumar B. Phytochemical analysis of high value medicinal plant *Valeriana jatamansi* using LC-MS and it's *in-vitro* anti-proliferative screening. Phytomed Plus 2021;1:100025.

89. Mohan C, Devi S. LC-MS and HPTLC profile of crude ethanol extract of plant *Michelia champaca* Linn. Pharmacogn Res 2024;16:490-4.

90. Tohma H, Gülçin İ, Bursal E, *et al.* Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. Food Measure 2017;11:556-66.

91. Wu L, Wu W, Cai Y, Li C, Wang L. HPLC fingerprinting-based multivariate analysis of phenolic compounds in mango leaves varieties: Correlation to their antioxidant activity and *in silico* α-glucoidase inhibitory ability. J Pharm Biomed Anal 2020;191:113616.

92. Liang W, Chen W, Wu L, Li S, Qi Q, Cui Y, *et al.* Quality evaluation and chemical markers screening of *Salvia miltiorrhiza* Bge. (Danshen) based on HPLC fingerprints and HPLC-MS(n) coupled with chemometrics. Molecules 2017;22:478.

93. Prasanth D, Reddy LS, Dasari T, Bhavanam PR, Ahmad SF, Nalluri R, *et al.* LC/MS-based profiling of *Hedyotis aspera* whole-plant methanolic extract and evaluation of its nephroprotective potential against gentamicin-induced nephrotoxicity in rats supported by *in silico* studies. Separations 2023;10:552.

94. Dwivedi MK, Sonter S, Mishra S, Singh P, Singh PK. Secondary metabolite profiling and characterization of diterpenes and flavones from the methanolic extract of *Andrographis paniculata* using HPLC-LC-MS/MS. Future J Pharm Sci 2021;7:1-28.

95. Abu Bakar FI, Abu Bakar MF, Abdullah N, Endrini S, Fatmawati S. Optimization of extraction conditions of phytochemical compounds and anti-gout activity of *Euphorbia hirta* L. (Ara Tanah) using response surface methodology and liquid chromatography-mass spectrometry (LC-MS) analysis. Evid Based Complement Alternat Med 2020;2020:4501261.

96. Singh PK, Singh J, Medhi T, Kumar A. Phytochemical screening, quantification, FT-IR analysis, and in silico characterization of potential bio-active compounds identified in HR-LC/MS analysis of the polyherbal formulation from Northeast India. ACS Omega 2022;7:33067-78.

97. Mabasa XE, Mathomu LM, Madala NE, Musie EM, Sigidi MT. Molecular spectroscopic (FTIR and UV-Vis) and hyphenated chromatographic (UHPLC-qTOF-MS) analysis and *in vitro* bioactivities of the Momordica *balsamina* leaf extract. Biochem Res Int 2021;2021:2854217.

98. Shaheen G, Ashfaq A, Khawar A, Jamil QA, Parveen R, Hadi F, *et al.* Fourier transform infrared spectrometer analysis and antimicrobial screening of ethanolic extract of *Operculina terpathum* from cholistan desert. Pharm Pract (Granada) 2022;20:2647.

99. Burman S, Bhattacharya K, Mukherjee D, Chandra G. Antibacterial efficacy of leaf extracts of *Combretum album* Pers. against some pathogenic bacteria. BMC Complement Altern Med 2018;18:213.

100. Baeshen NA, Almulaiky YQ, Afifi M, Al-Farga A,

Ali HA, Baeshen NN, *et al*. GC-MS analysis of bioactive compounds extracted from plant *Rhazya stricta* using various solvents. Plants (Basel) 2023;12:960.

101. Konappa N, Udayashankar AC, Krishnamurthy S, Pradeep CK, Chowdappa S, Jogaiah S. GC-MS analysis of phytoconstituents from *Amomum nilgiricum* and molecular docking interactions of bioactive serverogenin acetate with target proteins. Sci Rep 2020;10:16438.

102. Thamer FH, Thamer N. Gas chromatography - Mass spectrometry (GC-MS) profiling reveals newly described bioactive compounds in *Citrullus colocynthis* (L.) seeds oil extracts. Heliyon 2023;9:e16861.

103. Olivia NU, Goodness UC, Obinna OM. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. Future J Pharm Sci 2021;7:1-5.

104. Wen H, Yang M, Xu Z, Yang T, Zhang J. Characterization of aroma composition of *Amomum tsaoko* during the drying process based on GC-MS. Food Sci Nutr 2025;13:e4726.

105. Starlin T, Prabha PS, Thayakumar BK, Gopalakrishnan VK. Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. Bioinformation 2019;15:425-9.

106. Jabbar AA, Abdullah FO, Abdulrahman KK, Galali Y, Sardar AS. GC-MS analysis of bioactive compounds in methanolic extracts of *Papaver decaisnei* and determination of its antioxidants and anticancer activities. J Food Qual 2021;2022:1405157.