REVIEW
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HPTLC-MS: an advance approach in herbal drugs using fingerprint spectra and mass spectroscopy

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Author contributions

Dr. Alok conceived the original idea of the manuscript, whereas Ms. Babita has done extensive literature search using various searching tools. Dr. Alok and Ms. Babita wrote the manuscript. MS. Babita prepare the theoretical framework of this manuscript and Mr. Atiqul assisted in deriving figures and tables. All authors provided critical feedback and guided in the compilation and data analysis of the manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

MS, mass spectrometry; TLC, thin-layer chromatography; HPTLC, high-performance thin-layer chromatography; HRMS, high-resolution mass spectrometry; GC, gas chromatography; CE, capillary electrophoresis; LC, liquid chromatography; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; ESI, electrospray ionization; MALDI-TOF, matrix aided laser desorption/ionization time-of-flight.

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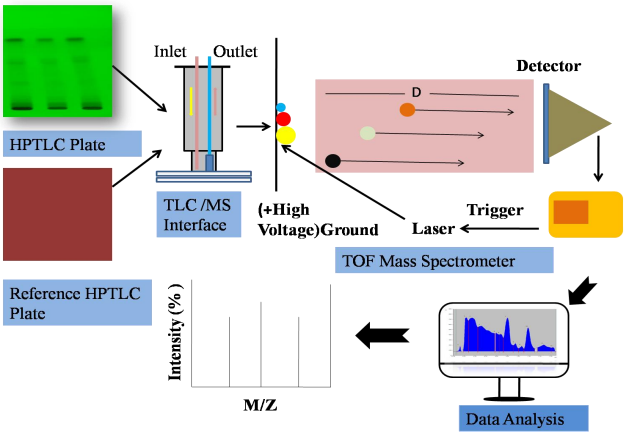
Abstract

High-performance thin-layer chromatography-mass spectrometry (HPTLC-MS) is one of the most modern hyphenated analytical methods available today. HPTLC-MS has recently been used to perform extensive analytical work using advanced features and still more analyses are in progress using these advanced features. Herbal medicines contain a wide range of bioactives that require proper identification for the activity and quality control. Recently, herbal medicine identification and quality control have become increasingly popular using high-performance liquid chromatography mass spectrometry. The aim of this paper is to provide a brief overview of recent method developments in analysis, 15 most significant herbal drug applications with their chromatographic conditions, pharmacological actions, and solvents used in the present paper. An extensive literature search was performed incorporating several databases, notably, Web of Knowledge, PubMed and Google Scholar, and other relevant published materials. In previous research, HPTLC and its hyphenation with MS allowed for quantification of analytes in complex matrixes at nanogram and picogram concentrations. Quantifying a wide variety of analytes using these techniques has been extremely accurate, selective, and sensitive. Throughout this review, the HPTLC-MS technique is discussed in relation to the quality control of herbal drugs. Hence, 15 herbal drugs were identified based on their RF values and m/z ratio by mass spectrometry by HPTLC-MS for the first time. In this compilation, researchers can gain insights into HPTLC-MS techniques for resolving quality control issues with herbal drugs using their fingerprint spectra. Besides, the application of HPTLC-MS methods could to be sufficiently precise and reproducible for established conditions and after validation may be used for routine quality control of herbal drugs/formulations in herbal industries.

Keywords: chromatography; herbal drug; HPTLC-MS; mass spectrometry; quality control



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Highlights

(1) Fifteen herbal drugs along with their chromatographic condition hyphenated with Mass spectrometry.
(2) Pharmacological activity of leading compound.
(3) Identification of selected compounds based on their Rf value and mass to charge ratio.
(4) Sample preparation method for various herbal drugs with different solvent system.

Medical history of objective

Crude drugs, medicinal plants and traditional formulations have been part of human civilization since centuries. Oldest civilizations used plant parts in the form of their extracts as food, medicine, and in different formulations. Various plant parts are the most commonly used to extract the bioactive compounds. These bioactives and extracts derived from medicinal plants which are used as drugs and responsible for various pharmacological activities. Plant extracts are complex and it is a mixture of diverse bioactives which need to be separated. High-performance thin-layer can analyze mixture and confirm the active chemical constituents. Recently, introduction of high-performance thin-layer mass spectrometry could be most effective tools to determine active constituents of crude drugs and herbal formulation. Besides, it can play an important role in the identification, separation and quality control of medicinal plants.

Background

Over time, the relevance of chromatography with mass spectrometry (MS) in analytical sciences has grown to the point that it is now considered vital, regardless of whether specific practical challenges or more fundamental issues are being addressed. In herbal drug analysis, chromatography is a useful analytical technique that has grown in importance and popularity in combination with its precedent significance [1]. In qualitative and quantitative research, it is an essential biophysical method to identify, quantify, separate and purify the components of a mixture [2]. Analyses such as these are critical especially in fields such as herbal drugs and phytopharmaceuticals. As a result, reliable analytical procedures are required to ensure the quality of herbal medications. However, even when conventional analytical techniques satisfy this requirement in a satisfactory manner, many analytical techniques require nanogram or picogram quantities of the substance to be analysed and in such cases, the quantitative analysis gets hard to perform [3]. These issues are resolved by hyphenated separations, which are reputable separation methods that separate components from mixtures efficiently and identify compounds using spectral methods. The high-performance thin-layer chromatography (HPTLC) approach has become one of the most adapted strategies and techniques to achieve significant milestone accomplishments in quantitative analysis [4]. It is simple, fast, and efficient. HPTLC, which has proven to be an easy, quick, as well as fast method for quantitative analysis, has recently emerged as a technology leading to many milestone achievements. To determine natural products from medicinal plants, an analytical method must meet high standards of separation quality, selectivity, and sensitivity [5]. The matrices are often complex, not only do they contain related compounds in trace amounts, but they are often followed by others that have similar structures.

HPTLC is a sensitive and sophisticated way to compare a sequence of samples to a reference material and fingerprint analysis, which produces specific and accurate results [1]. Traditionally, separation was carried out using HPTLC or thin-layer chromatography (TLC) techniques, followed by removal and identification of the separation material with MS [6]. Thus, in order to improve sensitivity, chromatographic systems have also been coupled to mass spectrometers as detectors, known as hyphenated techniques (for example, as HPTLC-MS, HPLC-MS, GC-MS, MS-MS, etc). HPTLC-MS is a rapid and sensitive technique for separating and identifying compounds from a mixed sample within a short timeframe ensuring specific detection of compounds down to the nanogram level and producing high purity results [7]. With this technique, questioned zones are placed into a mass spectrometer for identification, providing sensitive mass spectrometric data within less than one minute.

This study aims to review HPTLC-MS research on a more practical level, i.e., by individual herbal medicinal compounds and their applications. Besides providing a brief overview of recent technological developments, it includes a list of 15 most significant herbal drug applications with their chromatographic conditions, pharmacological actions, and solvents used in the extraction process. HPTLC-MS is extensively described in light of the current position of herbal drugs analysis by presenting important quality methodological information or discussing advantages and limitations of selected herbal drugs. It will provides straightforward information about effects arising from individual compounds in complex or natural samples separated in parallel. Moreover, this will helps to select from the thousands of compounds in a sample the important ones that need to be further characterized using high-resolution mass spectrometry (HRMS). Graphical abstract shows the schematic representation involved in analysing the natural products related to HPTLC-MS.

HPTLC-MS versus other analytical technique

In recent years, hyphenated procedures have received a lot of attention as the primary way to handle complicated analytical problems. Over the years, the power of integrating separation technologies with spectroscopic techniques for both quantitative and qualitative identification of unknown chemicals in complex natural product extracts or fractions has been established [8]. To obtain structural information leading to the identification of the compounds present in a crude sample, TLC/HPTLC, liquid chromatography (LC), usually a high-performance liquid chromatography (HPLC), gas chromatography (GC), or capillary electrophoresis (CE) is linked to spectroscopic detection techniques, e.g., Fourier-transform infrared, photodiode array UV-Vis absorbance or fluorescence emission, MS, and nuclear magnetic resonance (NMR) spectroscopy, resulting in the introduction of various modern hyphenated techniques, e.g., CE-MS, GC-MS, LC-MS, and LC-NMR [9, 10].

LC-MS

HPLC is the most widely used analytical separation technique for the qualitative and quantitative determination of compounds in natural product extracts. LC-MS or HPLC-MS refers to the coupling of an LC with a mass spectrometer. A typical automated LC-MS system includes a double three-way diverter in parallel with an auto sampler, an LC system, and a mass spectrometer. In general, the diverter acts as an automatic switching valve, diverting unwanted components of the eluate from the LC system to waste before the sample enters the MS. An LC-MS combines the chemical separation capacity of LC with the ability of an MS to identify and confirm molecular identity selectively. In general, the ionisation techniques utilised in LC-MS are soft ionisation techniques that primarily reveal molecular ion species with only a few fragment ions. As a result, the information received from a single LC-MS run on the compounds structure is quite limited [11, 12].

GC-MS

GC-MS, a hyphenated technology created by combining GC with MS, was the first of its kind to be helpful for research and development. Based on fragmentation interpretation, mass spectra acquired using this hyphenated approach provide more structural information. In GC-MS, a sample is injected into the injection port of GC device, vaporized, separated in the GC column, analyzed by MS detector, and recorded. The period between injection and elution is referred to as “retention time” (tR). In general, GC-MS equipment consists of an injection port at one end of a metal column (typically packed with

sand-like material to facilitate maximal separation) and a detector (MS) at the other end of the column [8, 13].

HPTLC-MS

HPTLC is an important alternative method to HPLC or GC due to the use of modern apparatus such as video scanners, densitometers, and new chromatographic chambers, as well as more effective elution techniques, high-resolution sorbents with selected particle size or chemically modified surface, the ability to combine with other instrumental methods such as MS, and the development of computer programmes for method optimization [14]. Hyphenating HPTLC with MS appears to hold considerable promise for those analysts who previously have had reservations towards the use of planar chromatography. HPTLC-MS, in particular, is a useful TLC technique for analytical purposes due to its increased accuracy, reproducibility, and ability to document results when compared to other techniques [15]. A study by Jautz et al. found that HPTLC-MS by a plunger-based extraction device was shown to be an appropriate technique for quantitative planar chromatography, even in trace analysis. Additionally, reproducible extraction from silica gel phases in the lower-pg range distinguishes this technique from other approaches [15].

HPTLC-MS is still one of the most adaptable, dependable, and cost-effective separation techniques for the investigation of botanicals and herbal medications. It ensures reproducible findings when used with standardized processes, which is critical in the routine identification of complex fingerprints of plant extracts and pharmaceutical products. The usage of HPTLC-MS is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC-MS remains one step ahead when compared with other tools of chromatography.

Recent technological development

HPTLC with matrix aided laser desorption/ionization time-of-flight (MALDI-TOF) MS

Over the past few years, many other papers have been published that used TLC paired with MS to gather analytical data on separated compounds directly from the TLC plates. Similar to LC, TLC is a substance-preservation chromatographic technology which preserves samples in a silica stationary phase. After the producing solvent evaporates, samples can be used for further research [16]. Another type of MS is MALDI-TOF-MS which uses a matrix to aid in desorption and ionization. Although MALDI-TOF MS is commonly used to analyse flavonoids, HPTLC-MALDI MS has been successfully used to analyse a variety of phytochemical mixtures. HPTLC (co-elution) and MALDI MS (fragmentation) can also cause issues during analysis when used alone. Combining these two techniques has proven to be an effective method of addressing these issues. Combining the standard and a low-cost TLC fingerprint with MALDI-TOF MS suggests that visible silica plate discoveries can be accompanied by ongoing mass offering data from the chromatographic pathway [17]. Furthermore, TLC plates co-eluting constituents are able to be differentiated from one another since the molecules' MS signals are not at the same m/z [18]. A TLC separation prior to MALDI MS will allow for the distinct identification of compounds consisting of the same m/z value (for example, luteolin and kaempferol aglycones, having the same m/z value). In HPTLC-MALDI MS chromatograms, their signals may appear at different heights (RF values). This is only true if they do not elute together because of chromatographic conditions [17]. Additionally, TLC-MALDI MS coupling provides the advantage that it eliminates the need to identify and choose analyte zones of interest on the plate. The TLC plate must have a physical attachment for each zone, prior to conducting research to link it with a crossing point. On the other hand, TLC coupled with MALDI-TOF MS uses the silica layer during chromatography to obtain MS information about a compound [18].

Applications of hyphenated HPTLC-MS technique in Chinese herbal drug analysis

Phytochemical studies began from chromatography that opened the doors of the micromolecular world. Analysing herbal medicinal components and their primary as well as secondary metabolites using an HPTLC-MS combination has proven to be a quick but effective procedure. It is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability [19]. A green extraction method was examined to enhance terpene lactone yield while minimising ginkgolic acid yield from *Ginkgo biloba* L. leaves. An HPTLC-MS approach for identifying and validating terpene trilactones and ginkgolic acids in *Ginkgo biloba* samples was developed and validated. The detection limits are 0.791, 0.850, 0.868, 0.785, 0.763, 0.871, and 0.622 μg/band, respectively, while the quantitation limits are 2.399, 2.576, 2.632, 2.380, 2.313, 2.640, and 1.885 μg/band for bilobalide, ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide K, ginkgol [20]. An HPTLC-MS method was developed for the effective screening of 11 chemical dyes (Sudan I, II, III, and IV; 808 Scarlet; Sudan Red 7B; malachite green; Basic Orange 2; auramine; Orange II; and erythrosine) in traditional Chinese medicine raw materials and Chinese patent medicines, with Sudan I and IV, 808 Scarlet, and Orange II successfully detected in eight batches of traditional Chinese medicine raw materials and Chinese patent medicines [21]. The methanol extracts of dietary supplements were evaluated on silica gel plates after manual sample application using n-hexane – ethyl acetate – ethanol (16:3:1, v/v/v) as a mobile phase. The chromatograms were quantified by scanning them in the absorbance mode at 290 nm. The limits of detection and quantitation were 90 and 280 ng/zone for magnolol and 70 and 200 ng/zone for honokiol, respectively [22]. The analytical profile and pharmacological activity of for various herbal drugs and its leading compounds with the different solvent system have been discussed in Table 1, Table 2, and Table 3. Few of the essential applications of hyphenated HPTLC-MS technique in herbal drug analysis have been discussed in this section with pictorial representation in Figure 1.

Table 1 Reported herbal drug along with their analytical profile through HPTLC MS hyphenation technique

No.	Herbal drug	Stationary phase	Mobile phase	Flow rate (mL/min)	Injection volume	Temp. (°C)	λ max	Target analytes	Detector	Ref.
1.	*Allamanda cathartica*	Silica gel 60 F254	Chloroform:methanol (9.5:0.5)	0.1	5 μL	105	540 nm	Iridoid glycoside	ESI-MS	[23]
2.	*Cannabis sativa*	Silica gel 60	N-hexane/acetone/triethylamine (40:20:2)	0.2	25 μL	250	254 nm	Cannabinol	ESI-MS	[29]
3.	*Curcuma longa*	Silica gel 60 F254	Toluene – ethyl acetate – formic acid (9:6:0.4)	0.1	Curcumin 0.2 μg/μL	250	366 nm	Curcumin	Single-quadrupole mass spectrometer	[35]
4.	*Cyclanthera pedata*	Amino without F254S	Ethyl acetate – formic acid – acetic acid water (30:1.5:1.5:3)	0.2	15 μL	240	254 and 366 nm	Apigenin 6-C-glucoside (isovitexin) and luteolin 8-*C*-glucoside (orientin)	ESI-MS	[41]
5.	*Fallopia japonica*	Silica gel 60	toluene – acetone – formic acid (3:6:1)	0.2	2 μL	200	280 nm	Flavan-3-ols and B-type proanthocyanidins	ESI-MS	[47]

Table 1 Reported herbal drug along with their analytical profile through HPTLC MS hyphenation technique (*continued*)

No.	Herbal drug	Stationary phase	Mobile phase	Flow rate (mL/min)	Injection volume	Temp. (°C)	λ max	Target analytes	Detector	Ref.
6.	Flavonoids (Propolis)	Silica gel 60 F254	N-hexane – ethyl acetate – formic acid (20:19:1)	0.2	5–22 µL	200–350	366 nm	Flavonoids (flavone, apigenin, luteolin, chrysin, quercetin dihydrate, myricetin, kaempferide, kaempferol, naringenin, pinocembrin)	ESI-MS	[51]
7.	*Hedera helix*	Silica gel 60F	Ethyl acetate:methanol:water:acetic acid (9:1:1:0.25)	0.25	4 µL	105	366 nm	Six phenolics; caffeic acid, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, kaempferol-3-O- rutinoside, rutin and chlorogenic acid and the three saponins; alpha-hederin, hederasaponin B and hederacoside C	ESI-MS	[54]
8.	*Helianthus annuus*	Silica gel 60 F254	N-hexane – isopropyl acetate – acetic acid (80:19:1, v/v)	0.1	5–20 µL	270	254 and 365 nm	(-)-Kaur-16-en-19-oic acid and 15-α-angeloyloxy-ent-kaur-16-en-19-oic acid	ESI-HRMS	[61]
9.	*Ilex paraguariensis*	Silica gel 60 F254	Dichloromethane and methanol (92:8) for methylxanthines; Ethyl acetoacetate, toluene, formic acid and H_2O (8.7:1.3:1.7:0) for phenolic compounds; ethyl acetoacetate toluene, formic acid and H_2O (9:1:2.5:1) for saponins	0.1	Caffeine (22.4 µg/mL); theobromine (4 µg/mL); rutin (8 µg/mL); 5-caffeoylquinic acid (80 µg/mL); and 3,5-dicaffeoylquinic acid (63 µg/mL).	200	274 nm for methylxanthines, 330 nm for phenolic compounds and 354 nm for rutin.	Methylxanthines, phenolic compounds, and saponins	ESI-MS	[64]
10.	*Physalis alkekengi*	Silica gel 60	Ethyl acetate – toluene – formic acid (7:3:0.2)	0.2	2 µL	280	366 nm	Physalins	Triple quadrupole-ESI	[72]
11.	Propolis	Silica gel	Methanol:water (60:40)	0.1	10 µL	100	254 and 365 nm	Pinobanksin, Pinocembrin	TOF mass spectrometer	[76]
12.	*Salvia miltiorrhiza*	Silica gel 60 F254	Toluene – chloroform – ethyl acetate – methanol – formic acid (4:6:8:1:1)	0.1	20–1000 ng/µL	270	254 nm and 366 nm	1,2-Dihydrotanshinone and methylenetanshinquinone	Quadrupole-Orbitrap mass spectrometer	[78]
13.	*Sambucus nigra*	Silica gel 60 with and without F254	Ethyl acetate – 2-butanone – formic acid – water in (5:3:2:1)		1–2 µL (fresh sample) and 2–20 µL (dried sample)	250	540 nm and 560 nm	Anthocyanins	ESI-MS	[83]
14.	*Silybum marianum*	Silica gel 60 F254	Toluene – ethyl acetate – formic acid (9:6:0.4)	0.1	Silibinin 0.4 µg/µL	250	366 nm	Silibinin	Single-quadrupole mass spectrometer	[35]
15.	*Zingiber officinale*	Silica gel 60 F254	Ethyl acetate, water – methanol (1:1), petroleum ether – t-butyl methyl ether (1:1).	0.1	2, 7.5, 15 µL	250	580 nm	Gingerol	Quadrupole-Orbitrap mass spectrometer	[92]

ESI-MS, electrospray ionization mass spectrometry; HRMS, high-resolution mass spectrometry; TOF, time-of-flight.

Table 2 Herbal drug and it leading compound having Pharmacological activity

No.	Herbal drugs	Major compounds	Leading compounds	Pharmacological activity	Mechanism of action	Ref.
1.	*Allamanda cathartica*	Allamandin, allamdin, allamcin, plumericin, isoplumericin, lactones, ursolic acid, β-amyrin, β-sitosterol and triterpenes	Allamcin, plumerides, allamandin, plumericin/isoplumericin and allamdin	Human carcinoma, human immunodeficiency virus, jaundice and malaria	In vitro studies against human carcinoma cells of nasopharynx while in vivo ethanolic root extract activity against P-388 leukaemia in mice.	[24, 25]
2.	*Cannabis sativa*	(-)-THCA, cannabidiolic acid, (-)-Δ9-THC and cannabidiol	Cannabinol	Pain, multiple sclerosis, anorexia, nausea and vomiting, colitis, sleep disorders, anxiety, epilepsy, schizophrenia, Alzheimer's disease, Parkinson's disease	Cannabinoid compounds modulate the CB1, CB2 receptors and metabolism of the endocannabinoids system, AEA and 2-AG	[27]
3.	*Curcuma longa*	Turmeric-diphenylheptanoid curcumin, volatile oils, tumerone, atlantone and zingiberone.	Curcuminoids	Antioxidative, hepatoprotective, anti-inflammatory and anticancer properties.	Curcuminoids in inflammation by reduced immune response decreased neutrophil migration and increased barrier remodelling.	[31–34]
4.	*Cyclanthera pedata*	Apigenin 200-O-rhamnose-C-hexoside, apigenin 6-C-glucoside (isovitexin), apigenin-8-C-(6"acetyl)-b-D-glucopyranoside, chrysin 6-C-fucopyranoside, luteolin 8-C-glucoside (orientin)	Apigenin 6-C-glucoside (isovitexin), luteolin 8-C-glucoside (orientin)	Anti-inflammatory, anti-oxidative, hypoglycaemic, hypocholesterolaemic, anti-hypertensive, atherosclerosis, blood circulation problems and analgesic	Anti-oxidant activity as a free radical scavenging properties and significant reduction of serum cholesterol levels as hypocholesterolaemic activity.	[37–39]
5.	*Fallopia japonica*	Stilbenes (resveratrol, piceatannol hexoside, piceid), anthraquinones (emodin, emodin-O-hexoside, emodin-O-(acetyl)-hexoside and emodin-O-(6'-O-malonyl)-hexoside) and proanthocyanidins (dimers and tetramers)	Flavan-3-ols and B-type proanthocyanidins from monomers to decamers	Oxidative stress-related diseases, cardiovascular diseases, diabetes type 2, allelochemicals	It protects islet cells from destruction and diabetes by leukocyte migration inhibition.	[43–46]

Table 2 Herbal drug and it leading compound having Pharmacological activity (*continued*)

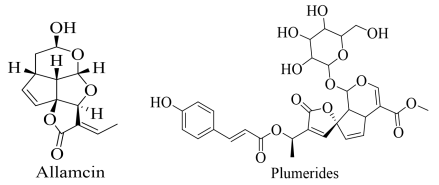
No.	Herbal drugs	Major compounds	Leading compounds	Pharmacological activity	Mechanism of action	Ref.
6.	Flavonoids (Propolis)	Phenolic and non-phenolic compounds	Flavonoid glycosides, polar flavonoid aglycones, non-polar flavonoid aglycones	Antioxidative, anti-inflammatory, immunomodulatory, antidiabetic, antilipidemic, antitumor, anti-aging, antimicrobial, antiviral and hepatoprotective	It act as free radical scavengers, inhibitors of specific enzymes and mimetics of certain neurotransmitters or hormones.	[54, 55]
7.	*Hedera helix*	Triterpene saponins, flavonoids, phenolic compounds, A-hederin and hederacoside C.	Caffeic acid, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside, rutin and chlorogenic acid and the three saponins; alpha-hederin, hederasaponin B and hederacoside C	Expectorant and broncho-spasmolytic activity, respiratory disorders	It suppresses the expression of *MUC5AC* gene, major mucin producing genes in chronic inflammatory airway diseases and showed bronchodilators activity	[57, 58]
8.	*Helianthus annuus*	Terpenoids, phenolics, flavonoids and coumarins.	(-)-Kaur-16-en-19-oic acid and 15-α-angeloyloxy-ent-kaur-16-en-19-oic acid	Anti-inflammatory and antimicrobial effects	It showed antibacterial effects against Gram-positive *Bacillus subtilis* and Gram-negative *Aliivibrio fischeri* bacteria.	[61–64, 93]
9.	*Ilex paraguariensis*	Caffeine, theophylline, theobromine, rutin, caffeoylquinic acid, dicaffeoylquinic acid, quercetin, kaempferol, phenolics and saponins	Methylxanthines, phenolics and saponins	Antioxidant, antimicrobial, antidiabetic, analgesic anticancer, central nervous system stimulant, diuretic, lipoxygenase-inhibitor, anti-ulcer	Poly phenols are reducing agents and provide body tissues protection from oxidative stress that causes aging, cancer, cardiovascular disease and inflammation. Saponins reported to provide a hypocholesteremic effect by inhibiting the passive diffusion of colic acid through the formation of micelles preventing absorption, anticancer, antiparasitic, and anti-inflammatory properties.	[68]
10.	*Physalis alkekengi*	Physalins, carotenoids, alkaloids, flavonoids, glucocorticoids, lycopene, ethanolic compounds, and vitamin C.	Physalins	Antibacterial, antitumor, anti-inflammatory, immunosuppressive, antinociceptive, antimalarial, leishmanicidal, antispermatogenic and antifertility.	Physalins decreased inflammation through decreasing neutrophils infiltration and inhibiting the formation of interleukin-6 and interleukin-12.	[70–73, 75]
11.	Propolis	Pinobanksin pinocembrin, chrysin galangin, phenolic acids and flavonols	Pinobanksin, pinobanksin 3-O-pentanoate, pinobanksin 2-methylbutyrate and caffeic acid	Antimicrobial, anti-inflammatory, immunomodulatory, antitumor and antidiabetic activities.	Propolis inhibits the growth of *Penicillium digitatum*, as well as inhibit the growth of microbial pathogens which shows anti-microbial activity.	[78–80]
12.	*Salvia miltiorrhiza*	Phenolic acidsi.eRosmarinic acid and tanshinones I, IIA, IIB and cryptotanshinone	Salvianolic acid B, lithiospermic acid rosmarinic acid, cryptotanshinone and 15,16-dihydrotanshinone I	Alzheimer's, Parkinson's, cerebrovascular, coronary heart diseases, cancer, renal deficiency, bone loss, hepatocirrhosis, gynaecologic disorders and skin lesions.	The antioxidant capacity by increasing activity of SOD and GPx and decreasing the level of MDA and ROS production significantly. The activity in cardiovascular disease by promoting microcirculation, dilates coronary arteries, enhances blood flow, prevents uptake and oxidation of low-density lipoprotein, and protects from ischemia-reperfusion injury.	[81, 82]
13.	*Sambucus nigra*	Cyanidin derivatives (cyanidin-3-glucoside, cyanidin-3-sambubio- side, and cyanidin-3-sambubioside-5-glucoside) flavonols (quercetin and kaempferol), phenolic acids (chlorogenic acid and its derivatives), procyanidinsterpenes and lectins, free and conjugated forms of amino acids, proteins, unsaturated fatty acids, fibre fractions as well as vitamins and minerals.	Anthocyanin content i.e. cyanidin 3-sambubioside (cn-3-sam) and cyanidin 3-glucoside (cn-3-glc) and some traces of pelargonidin, petunidin or delphinidin derivatives.	Diaphoretic, diuretic, viral infections (common cold and influenza), antiviral drug, diabetes, cardiovascular diseases cancer and antioxidant activity.	The antioxidant activity of Cy 3-glucoside on liposomal membranes as antiradical properties. The anti-inflammatory activity due to inhibition of the enzymes cyclooxygenase-1 and cyclooxygenase. The anticancer activity of Cy 3-gluc on human breast adenocarcinoma cell line.	[83]
14.	*Silybum marianum*	Silymarin, silibinin, isosilibinin, silicristin and silidianin	Silybinin	Anti-inflammatory, anticarcinogenic, hypocholesterolemic, immune modulators, congestion	Silybinin strengthens and stabilizes the cell membranes, inhibits the synthesis of prostaglandins associated with the lipid peroxidation and promotes regeneration of liver through the stimulation of protein synthesis and effect on the production of new hepatocytes.	[84, 94]
15.	*Zingiber officinale*	Zingiber, zingiberene gingerols, 8-gingerols, 10-gingerols, shogaols, essential oil as well as non-volatile oleoresin fraction.	6-Gingerol, 6-shogaol	Antioxidative, anti-inflammatory, cancer preventive, antimicrobial, antifungal, acetylcholinesterase inhibitory, positive effects on blood pressure, blood clotting, inflammation at gastrointestinal tract as well as antioxidant activity.	The anti-inflammatory activity mainly due to PI3K, Akt, and the nuclear factor kappa light chain-enhancer of activated B cells (NF-κB). The anti-microbial activity by inhibiting the growth of *Pseudomonas aeruginosa* by affecting membrane integrity and inhibiting bio film formation.	[91, 92, 95]

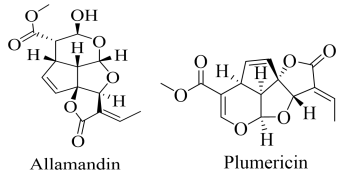
THCA, trans-tetrahydrocannabinolic acid; Δ 9-THC, Δ 9-trans-tetrahydrocannabinol; PI3K, phoshatidylinositol-3-kinase; Akt, protein kinase B; 2-AG, 2-arachidonoylglycerol; AEA, N-arachidonoylethanolamine;GPx, glutathione peroxidase; SOD, superoxide dismutase; ROS, reactive oxygen species; MDA, malondialdehyde.

Table 3 Sample preparation method for various herbal drugs with different solvent system

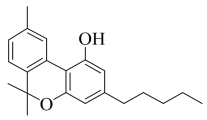
No.	Herbal drugs	Sample preparation method	Instrument name	m/z Ratio	Reported compounds	Mol. Wt.	Ref.
1.	*Allamanda cathartica*	Maceration followed by fractionation	CAMAG, Switzerland	Allamcin (250.250), plumerides (470.420), allamandin (309.365), plumericin & isoplumericin (290.270 & 290.070) allamdin (291.074)	Allamcin, plumerides, allamandin, plumericin/isoplumericin and allamdin	Allamcin(250.25 g/mol), plumerides (616.6 g/mol), allamandin (308.28 g/mol)	[23, 27]
2.	*Cannabis sativa*	Solid phase extraction	CAMAG, Switzerland	Cannabinol(309 and 354)and cannabinol-d3 (312 and 357)	Cannabinol, cannabinol-d3	Cannabinol (310.4 g/mol), cannabinol-d3 (313.45 g/mol)	[29]
3.	*Curcuma longa*	Mechanical grinding with centrifugation	CAMAG, Switzerland	Curcuminoids (391)	Curcuminoids	Curcuminoids (368.38 g/mol), silibinin (482.4 g/mol)	[35]
4.	*Cyclanthera pedata*	Lyophilisation and centrifugation	CAMAG, Switzerland	Isovitexin (431), orientin (447)	Apigenin 6-C-glucoside (isovitexin), luteolin 8-C-glucoside (orientin)	Apigenin 6-C-glucoside (564.5 g/mol), luteolin 8-C-glucoside (447.4 g/mol)	[41]
5.	*Fallopia japonica*	Solid phase extraction and counter current chromatography	CAMAG, Switzerland	B-type proanthocyanidins (289-1729)	Flavan-3-ols and B-type proanthocyanidins from monomers to decamers	Flavan-3-ols (226.27 g/mol), B-type proanthocyanidins (578.52 g/mol)	[47]
6.	Flavonoids (Propolis)	Pulverization, sonication and centrifugation.	CAMAG, Switzerland	Flavonoid glycosides (399-253), polar flavonoid aglycones (515-349), non-polar flavonoid aglycones (351-301)	Flavonoid glycosides, polar flavonoid aglycones, non-polar flavonoid aglycones		[51]
7.	*Hedera helix*	Ultrasonication	CAMAG, Switzerland	Caffeic acid (179.1), kaempferol-3-O-glucoside (447.1), quercetin-3-O-glucoside (463.1), kaempferol-3-O-rutinoside (595.1), rutin (609.1), alpha-hederin (749.2), hederasaponin B (1203.1), hederacoside C (1219)	Caffeic acid, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside, rutin and chlorogenic acid and three saponins; alpha-hederin, hederasaponin B and hederacoside C	Caffeic acid (180.16 g/mol), kaempferol-3-O-glucoside (448.4 g/mol), quercetin-3-O-glucoside (463.4 g/mol), kaempferol-3-O-rutinoside (594.5 g/mol), rutin (610.5 g/mol), chlorogenic acid (354.31 g/mol), alpha-hederin (751 g/mol), hederasaponin B (1205.4 g/mol) hederacoside C (1221.4 g/mol)	[54]
8.	*Helianthus annuus*	Maceration and flash chromatography fractionation.	CAMAG, Switzerland	(-)-Kaur-16-en-19-oic acid (301.21725) and 15-α-angeloyloxy-ent-kaur-16-en-19-oic acid (399.25381)	(-)-Kaur-16-en-19-oic acidand 15-α-angeloyloxy-ent-kaur-16-en-19-oic acid	(-)-Kaur-16-en-19-oic acid (302.5 g/mol), 15-α-angeloyloxy-ent-kaur-16-en-19-oic acid (302.5 g/mol)	[61]
9.	*Ilex paraguariensis*	Exhaustive aqueous extraction method	CAMAG, Switzerland	Theobromine (181), caffeine (195), phenolics (353), saponins (1219)	Methylxanthines, phenolics and saponins	Methylxanthines (152.11 g/mol), phenolics (94.11 g/mol), saponins (1223.3 g/mol)	[64]
10.	*Physalis alkekengi*	Ultrasound assisted extraction and reflux extraction	CAMAG, Switzerland	Physalins (529)	Physalins	Physalins (544.5 g/mol)	[67, 72]
11.	Propolis	Culture growth and shaking	CAMAG, Switzerland	Pinobanksin (271), pinobanksin 3-O-pentanoate (355), pinobanksin 2-methylbutyrate (355), caffeic acid (179)	Pinobanksin, pinobanksin 3-O-pentanoate, pinobanksin 2-methylbutyrate and caffeic acid.	Pinobanksin (272.25 g/mol), pinobanksin 3-O-pentanoate (328.3 g/mol), pinobanksin 2-methylbutyrate (101.12 g/mol), caffeic acid (180.16 g/mol)	[76]
12.	*Salvia miltiorrhiza*	Decoction, ultrasonication , centrifugation and dispersive liquid liquid extraction	CAMAG, Switzerland	Salvianolicacid B (717.14), lithiospermic acid (537.10), rosmarinic acid (359.07), cryptotanshinone (319.13), 15,16-dihydrotanshinone I (304.10)	Salvianolicacid B, lithiospermic acid rosmarinic acid, cryptotanshinone and 15,16-dihydrotanshinone I	Salvianolicacid B (718.6 g/mol), lithiospermic acid (538.5 g/mol), cryptotanshinone (296.4 g/mol), 15,16-dihydrotanshinone I (278.3 g/mol)	[78]
13.	*Sambucus nigra*	Homogenization and centrifugation	CAMAG, Switzerland	Cyanidin 3-sambubioside (581), cyanidin 3-glucoside (449).	Anthocyanin content i.e. cyanidin 3-sambubioside (cn-3-sam) and cyanidin 3-glucoside (cn-3-glc) and some traces of pelargonidin, petunidin or delphinidin derivatives.	Cyanidin 3-sambubioside (616.95 g/mol), cyanidin 3-glucoside (484.83 g/mol), pelargonidin (271.24 g/mol), petunidin(317.27 g/mol), delphinidin (338.69 g/mol)	[83]
14.	*Silybum marianum*	Mechanical grinding with centrifugation	CAMAG, Switzerland	Silibinin (481 and 301)	Silibinin	Silibinin (482.4 g/mol)	[84]
15.	*Zingiber officinale*	Extraction followed by continuous shaking and centrifugation.	CAMAG, Switzerland	6-Gingerol (317.17), 6-shogaol (349.24)	6-Gingerol, 6-shogaol	6-Gingerol (294.4 g/mol), 6-shogaol (276.4 g/mol)	[92]

1. *Allamanda cathartica*

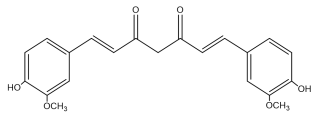




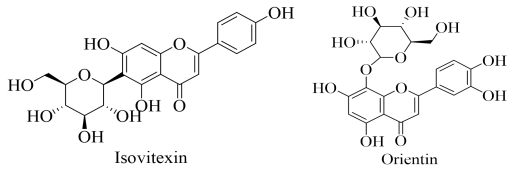
2. *Cannabis sativa*



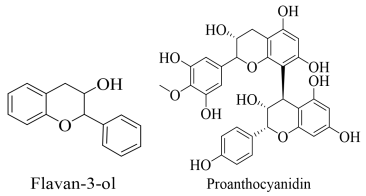
3. *Curcuma longa*



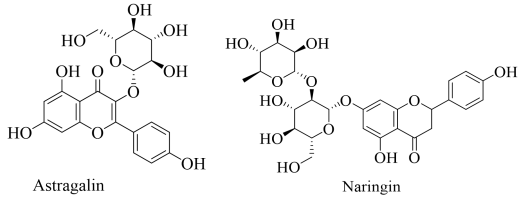
4. *Cyclanthera pedata*



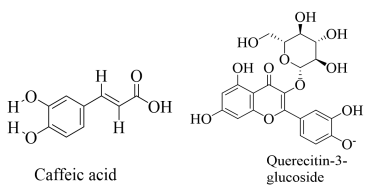
5. *Fallopia japonica*



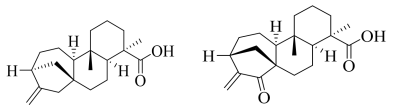
6. Flavonoids (Propolis)



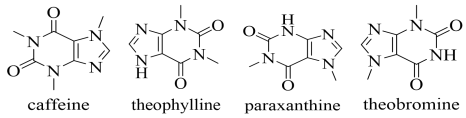
7. *Hedera helix*



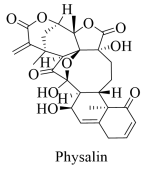
8. *Helianthus annuus*



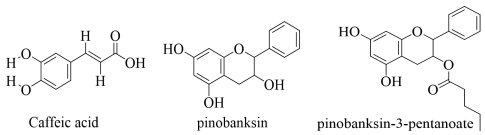
9. *Ilex paraguariensis*



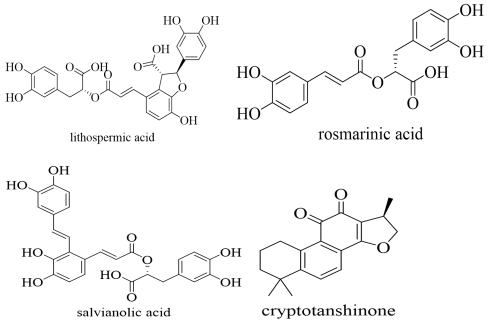
10. *Physalis alkekengi*



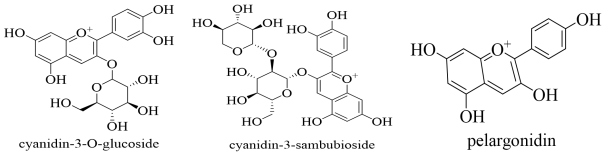
11. Propolis



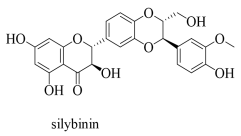
12. *Salvia miltiorrhiza*



13. *Sambucus nigra*



14. *Silybum marianum*



15. *Zingiber officinale*

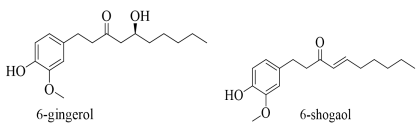


Figure 1 Chemical structure of different herbal drug which shows pharmacological activity

Identification of chemical constituents of *Allamanda cathartica*

Allamanda cathartica L., a flowering plant species belonging to family Apocynaceae is native to Brazil and was identified using HPTLC-MS [23]. Biologically, it contains iridoid lactones such as allamdin, allamdin, plumericin, and isoplumericin which prevent and treat human immunodeficiency virus and leukemia, and are used in the treatment of cancer, jaundice, and malaria [24]. Aside from lactones, ursolic acid, amyrin, and sitosterol, plant leaves and stems contain other compounds that are antimicrobial and astringent [25]. Mehrun Nisha Khanam et al. evaluated the synthesis and identification of iridoid glycosides in *Allamanda cathartica*. L using HPTLC-MS. During HPTLC-MS investigations, precoated silica gel 60 F254 was employed. Scanning was performed at 540 nm using a CAMAG TLC scanner III and winCATS 1.2.3 software with an MS detector and the bundled Mass Lynx v4.1 program. To determine the amount of iridoid glycoside, all samples and the control were run on an identical precoated TLC plate, and all 11 tracks at 540 nm could be seen. At this wavelength, several bands were recorded with varying RF values compared to the control sample and every 10 treatment samples. MS analysis identified the HPTLC bands at varying RF values as allamcin (0.23), plume rides (0.35), allamandin (0.63), plumericin (0.72), isoplumericin (0.72), and allamdin (0.82), respectively. Compounds were discovered to have fragment patterns in their first-order mass spectra, and these patterns are thought to be related to the fragment patterns of the compounds above. Allamcin (250.250), plumerides (470.420), allamandin (309.365), plumericin, and isoplumericin (290.270 and 290.270), and allamdin (290.270) were detected using a mass spectral library method and a comparison to a reference chemical (291.074). *Allamanda cathartica* was identified using HPTLC-MS based phytochemical investigations and demonstrated the presence of iridoids that could serve as substitute sources for iridoid glycosides and needed to be evaluated at a high level [23].

Identification and quantification of cannabinol by HPTLC-electrospray ionization (ESI)-MS

Cannabis (*Cannabis sativa* L.), commonly called marijuana, is a flowering plant of the Cannabaceae family. It is widely distributed worldwide, mainly in countries like north-western China, Afghanistan, Canada, India, and Pakistan. Archaeological evidence indicates that it has been used around for over 2,500 years [26]. Phytocannabinoids, such as (-)-9-trans-tetrahydrocannabinolic acid, cannabidiolic acid, (-)-9-trans-tetrahydrocannabinol, and cannabidiol are chemical compounds found mostly in female flora or mainly aerial sections of the cannabis herb [27]. The traditional medicinal use of the cannabis plant with a very long history, includes mainly intoxicant, analgesic, narcotic, stomachic, antispasmodic, anodyne, sedative properties. It has been pharmacologically used in the management of a large number of illnesses, such as discomfort, multiple sclerosis, anorexia, nausea and vomiting, colitis, sleep difficulties, Alzheimer's disease, Parkinson's disease [28]. Theresa Schmidt et al. demonstrated the use of HPLC-ESI-MS in detecting and quantifying cannabinol as a biomarker. HPTLC plates were used with a Linomat 5 applicator and developed in n-heptane/diethyl ether/formic acid (75:25:0.3 v/v/v) or n-hexane/acetone/triethylamine (40:20:2 v/v/v) using n-hexane/acetone/triethylamine (40:20:2 v/v/v). Developing chamber plates were examined under white light. To derivatize the samples, fetal bovine serum and cerium molybdenum reagent were used and compounds were eluted from HPTLC plates using a TLC-MS interface and a small mass spectrometer with an ESI ion source. When TLC or HPTLC plates were developed in n-hexane/diethyl ether/formic acid (75:25:0.3 v/v/v), n-hexane/diethyl ether (90:10 v/v), and n-hexane/acetone/triethylamine (40:20:2 v/v/ (0.6 and 0.8), RF values were (0.4 and 0.5) at 254 ˚C. Immediately post chromatographic separation using n-heptane/diethyl ether (90:10 v/v) with the developing solvent, cannabinol and cannabinol-d3 were detected as 309 and 312 using mass spectrometer spectra. Using this simple and quick approach, high-throughput, low-cost screening of sediment samples can be accomplished to reconstruct the history of cannabis retting [29].

Identification and quantification of bioactive components in turmeric using HPTLC coupled with (bio) assays and MS.

Turmeric (*Curcuma longa*) is a flowering plant belonging to ginger family Zingiberaceae and is commonly used in tropical Africa and Asia as a medicinal herb [30]. Turmeric rhizomes contains curcumin (diferuloylmethane), a linear diphenylheptanoid. It also contains volatile oils such as turmerone, atlante, and zingiberene which have antioxidative [31], hepatoprotective [32], anti-inflammatory [33], and anticancer [34] properties. In a separate study, Mahmoud N. Taha et al. identified and quantified bioactive components in turmeric using HPTLC and a single-quadrupole mass spectrometer. For HPTLC-MS analysis, silica gel 60 F254 HPTLC plates were used in the twin trough chamber (CAMAG) with the automatic TLC sampler and the solvent system toluene – ethyl acetate – formic acid 9:6:0.4 (v/v/v) at a temperature of 25 ˚C. The chromatograms were documented using the TLC visualizer (CAMAG) and scanned between 200 and 700 nm using the TLC Scanner 3 and winCATS software (CAMAG). In addition to the TLC-MS interface (CAMAG), the ESI interface was utilized to record the mass spectra. For three consecutive bioassays of turmeric in ethanolic extracts, the HPTLC-UV/Vis/FLD-bioactivity of 11 ethanolic herb extracts with solvent system combination made up of Toluene – ethyl acetate – formic acid 9:6:0.4 (v/v/v). For curcumin, hRF values of 57 were achieved by the mobile phase technique in only 20 minutes, which was able to resolve these marker chemicals. Additionally, HPTLC-UV/Vis/FLD measurements and quantification of specific compounds in turmeric were recorded to determine the best wavelengths or validate the compound and accurate identification by comparing spectra in between samples or standard zones. As the zones were eluted into the MS, complete scan mass spectra between m/z 100 and 1000 were plotted. Using positive ion analysis, both the traditional curcumin zone and the bioactive zone in turmeric exhibited the same mass signal at m/z 391. The hyphenated HPTLC-MS is a powerful approach for analyzing curcumin bioactive phytochemicals in *Curcuma longa*, so it is recommended for natural product analyses [35].

Analysis of flavonoids in *Cyclanthera pedata* using HPTLC and HPTLC-MS/MS

Cyclanthera pedata is a plant species native to Peru belonging to family Cucurbitaceae. It is grown as an edible plant in South American countries and a variety of environmental and pedo-climatic conditions are suitable for its growth [36]. The *Cyclanthera pedata* seeds consists of cucurbitacin glycosides [37] and triterpenoid saponins [38]; while fruits and leaves contains flavonoid glycosides [39] as the primary constituent. Its medicinal properties include cholesterol control, hypertension control, antioxidant action, anti-inflammatory properties, and inhibition of angiotensin converting enzymes [40]. To analyze flavonoids in *Cyclanthera Pedata*, Francesca Orsini et al. utilized HPTLC and HPTLC-MS/MS analytical techniques. HPTLC-MS chromatographic settings included HPTLC silica gel (preconditioned with water) or C18 plates with mobile phase ethyl acetate – water – formic acid (17:3:2, v/v) or 5% formic acid in methanol-water (7:3, v/v) and Linomat 5% formic acid in methanol-water (7:3, v/v) and Linomat 5% formic acid in methanol-water (CAMAG). In order to document the pictures and derivatization for flavonoids with natural product reagent, as well as to demarcate the chromatographic zones for future HPTLC-MS research, the plates were illuminated at 366 nm. Mass spectra was obtained with the ESI source and negative ion mode, using the TLC-MS interface (CAMAG) and components were eluted from the plates using an MS ion source. An HPTLC-DPPH assay was used in conjunction with image analysis and HPTLC-MS/(MS) analysis in this study, for direct analysis of antioxidant properties of distinct chromatographic zones (compounds) on silica gel plates. A flavonoid concentration in various zones of crude extracts was determined, and several active components were identified tentatively. Apigenin 6-C-glucoside (isovitexin) with RF value 0.45 and m/z ratio value 431, and luteolin 8-C-glucoside (orientin) with RF value 0.36 and m/z ratio value 447 were identified in all crude extracts [41].

Analysis of flavan-3-ols and proanthocyanidins in Japanese knotweed using HPTLC- MS

Polygonum cuspidatum (synonym, *Fallopia japonica* (Houtt), *Polygonum reynoutria*, and *Reynoutria japonica*) is a plant species belonging to family Polygonaceae. It is also known as Japanese knotweed. It is as an ornamental plant and is native to Eastern Asia which was brought to Europe in the 19th century [42]. It is among the top 100 invasive alien species in the world in Europe and North America. Its rhizomes contains trans-resveratrol, a stilbene which is in abundance [43], anthraquinone emodin [44], procyanidin dimers [45], flavan-3-ol tetramers, and monomeric units. It is used in traditional Chinese medicine to treat various conditions such as arthritis, diuretic difficulties, diarrhoea, cardiovascular disorders, and type 2 diabetes [46]. Vesna Glavnik et al. quantified flavan-3-ols and proanthocyanidins in Japanese knotweed using HPTLC with a MS. HPTLC silica gel and HPTLC silica gel MS grade plates were employed in an unsaturated twin-trough chamber for HPTLC-MS analysis (CAMAG) and developing solvents toluene-acetone-formic acid (3:3:1, 6:6:1, 3:6:1, v/v) and dichloromethane-acetone-formic acid (1:1:0.1, v/v) were used. Following drying, CAMAG immersion device III was used to immerse generated plates. Post-chromatographically, the product was derivatized using the 4-dimethylaminocinnamaldehyde detection reagent. For taking images of chromatographic plates under UV light (366 nm) before and after derivatization, a CAMAG digistore 2 documentation system was used with repro star 3 technology. White light illumination was scanned with the CAMAG TLC scanner 3, which was controlled by the winCATS program. The mass spectra were obtained by using an LCQ ion trap apparatus with Xcalibur 1.3 software and a negative ion ESI ion source, and the RF values for proanthocyanidins on HPTLC silica gel plates twice predeveloped with dissimilar parameters. The RF values of trimers (0.19, 0.29, 0.52, 0.32), trimer gallates (0.19, 0.29, 0.52, 0.28), tetramers gallates (0.10, 0.21, 0.35, 0.11), pentamers (0.04, 0.15, 0.35, 0.06), pentamer gallates (n.d, 0.15, 0.28, 0.03), hexamers (n.d, 0.07, 0.28, 0.02), hexamer gallates (n.d, 0.07, 0.28, 0.02), heptamers (n.d, 0.05, 0.19, n.d), octamers (n.d, n.d, 0.16, n.d), nonamers (n.d, n.d, 0.10, n.d), decamers (n.d, n.d, 0.05, n.d) and the MS spectra with m/z value of the above compounds were (865, 1017, 1305, 1441, 1593, 1729, 940, 1008, 1152 and 1440). A significant benefit of this method was its ability to identify both flavan-3-ol and proanthocyanidin contents in Japanese knotweed, and it is vital for developing methods for analyzing complicated proanthocyanidin combinations in crude extracts using HPTLC-MS [47].

Analysis of flavonoids using HPTLC-MS

A flavonoid is a secondary metabolite synthesized from phenylalanine and a derivative of benzo-γ-pyrone (chromone) [48]. Fruits and vegetables contain flavonoids, which are pigments and cell development regulators. Flavonoids have immunomodulatory, antidiabetic, antilipidemic, antitumor, anti-aging, antibacterial, antiviral, hepatoprotective, free radical scavengers, enzyme inhibitors, and neurotransmitter or hormone inhibitors properties [49, 50]. Urska Jug et al. utilized HPTLC-MS procedures using Linomat 5 (CAMAG) and without the fluorescence indicator F254 on silica gel plates to study flavonoids (roasted coffee, rosehip, hibiscus, rosemary, sage, and propolis). To develop the plates, n-hexane-ethyl acetate formic acid (20:19:1, v/v/v) was used as a developing solvent. The TLC-MS crossing point (CAMAG) served as a method for eluting chemicals into the mass spectrometer and was used in documenting developed plates at 366 nm for the ionization of compounds. In the m/z range of 50–1000, the spectra were obtained using hot ESI in negative ion mode. The phenolic components provisionally discovered in propolis samples using HPTLC-ESI-MS, together with their m/z and RF value were coumaroyl caffeoyl glycerol (399, 0.13), feruloyl caffeoyl glycerol (429, 0.13), caffeoyl coumaroyl acetyl glycerol (441, 0.25), dicoumaroyl acetyl glycerol (425, 0.34), iso ferulic acid (193, 0.34), coumaric acid isomers (163, 0.34, 0.36), naringenin (271, 0.36), caffeic acid benzyl ester (269, 0.36), genkwanin (283, 0.40), vanillin (151, 0.40), kaempferide (299, 0.44), iso-sakuranetin (285, 0.47), *p*-coumaric benzyl ester (253, 0.52), 3,5-O-dicaffeoyl quinic acid (515, 0.00), quinic acid a (191, 0.00), 3-caffeoyl quinic acid/5-caffeoylquinic acid (353, 0.00), feruloyl quinic acid (367, 0.03), caffeoyl quinic-1,5-lactone are the phenolic and non-phenolic compounds tentatively identified in roasted coffee samples through HPTLC-ESI/MS analysis with (335, 0.06), dicaffeoyl shikimic acid (497, 0.06), malic acid a (133, 0.06), 3-feruloyl quinic-1,5-lactone (349, 0.10). This method was fast, specific, and provided good selectivity for analysing flavonoids which made it an effective approach for identifying flavonoids throughout various matrices [51].

Targeted and untargeted chemical profiling of *Hedera helix* using HPTLC-MS

Hedera helix, a leaf, commonly known as Ivy, and belonging to the Araliaceae family has been used as a traditional medicinal herb since the 19th century [52]. The chemical analysis revealed active chemical elements such as triterpene saponins, flavonoids, and phenolic substances [53]. It has traditionally been used to treat respiratory problems due to its expectorant and broncho-spasmolytic properties. Eman Shawky et al. concluded the HPTLC-MS analysis of the *Hedera helix* for both targeted and untargeted chemical profiling. Samples and standard solutions were administered on HPTLC silica gel 60F plates with the aid of an automated Linomat V (CAMAG) applicator and ethyl acetate: methanol:water:acetic acid (9:1:1:0.25 v/v/v/v), and ethyl acetate: methanol:water:acetic acid (20:5:4:0.5 v/v/v/v) as a developing solvent in twin trough chamber were utilized and controlled using winCATS manager software (CAMAG). To identify chemical markers, HPTLC-PCA untargeted secondary metabolite analysis was used to determine the chemical fingerprinting profile of the various *Hedera helix* subspecies. The RF values and m/z ratio of six phenolics chemical markers were caffeic acid (0.45, 179.1), kaempferol-3-O-glucoside (0.66, 447.1), quercetin-3-O-glucoside (0.67, 463.1), kaempferol-3O-rutinoside (0.75, 595.1), rutin (0.77, 609.1), and chlorogenic acid (0.84) which were designated as (system I) as well as the RF values and m/z ratio of three saponins chemical markers were alpha-hederin (0.78, 749.2), hederasaponin B (0.41, 1203.1) and hederacoside C (0.37, 1219.0) designated as (system II). HPTLC-MS was used to identify the samples. This method was the first attempt at differentiating among the subspecies of *Hedera helix*, and it revealed that HPTLC-MS was suitable for chemical profile analysis of *Hedera helix* leaf constituents based on the holistic efficacy-related profile [54].

Bioanalytical profiling of sunflower leaves using hyphenated HPTLC

Sunflowers (*Helianthus annuus* L.) are from Asteraceae family, a family of annual wildflowers indigenous to North America that is extensively cultivated for seed and seed oil. Allelopathy is a vital defence mechanism caused by secondary metabolites [55]. Terpenoids [56], phenolics, flavonoids [57], and coumarins [58] are abundant in the aerial portion of the plant. These have biological qualities such as anti-inflammatory [59] and anti-microbial properties [60]. Agnes M. Moricza et al. demonstrated HPTLC with a single quadrupole MS for bio-analytical profiling of sunflower leaves. In the hyphenated technique chromatographic conditions, HPTLC silica gel 60 F254 plates with TLC sampler Linomat IV (CAMAG) and n-hexane–isopropyl acetate – acetic acid (80:19:1, v/v) as a developing solvent in twin trough chamber were utilized (CAMAG). Plates were dried and recorded under UV and white light by the TLC visualizer documentation system (CAMAG), which was then processed by winCATS software, following derivatization with primuline reagent (1:1, v/v) or vanillin-sulphuric acid reagent by immersion in the TLC immersion device (CAMAG) and heating at 110 ˚C for 5 minutes. Following this, active chemicals were characterized using HPTLC-ESI-HRMS and HPTLC-(direct analysis in real-time)-MS/MS. Based on this approach, two diterpenes compounds were found to have antibacterial activities, (-)-kaur-16-en-19-oic acid and 15-angeloyloxy-ent-kaur-16-en-19-oic acid. These diterpenes were multi-potent inhibitors of acetylcholinesterase. Hyphenated method

was used to validate the m/z values of (-)-kaur-16-en-19-oic acid and 15-angeloyloxy-ent-kaur-16-en-19-oic acid (301.217, 399.253). This method was appropriate for the bioanalytical profile of sunflower leaf, and the first publication applied a hyphenated methodology to evaluate the antibacterial and cholinesterase inhibitory activities among sunflower leaf extracts [61].

Bioanalytical profiling of mate tea (*Ilex paraguariensis*) using HPTLC

The *Ilex paraguariensis* is also known as yerba mate or erva mate, a tree from South America whose dried and crushed leaves are often infused with water for medicinal purposes [62]. In the process of making it, there are several stages including harvesting, blanching, hot air drying, coarse milling, aging, and sieving [63]. Methylxanthines, phenols, and saponins are among the most active compounds found in the chemical components. Using HPTLC and a single quadrupole MS, Pedro Kaltbach et al. measured methylxanthines, phenolic compounds, and saponins in mate tea aqueous extracts. HPTLC silica gel 60 F254 plates with Automatic TLC sampler 4 were used to optimize chromatographic settings for novel HPTLC procedures (CAMAG) in automatic developing camber (CAMAG) with anisaldehyde-sulfuric acid reagent as the derivatizing agent, with solvent system dichloromethane:MeOH (92:8) for methylxanthines, EtOAc, toluene, formic acid, and H2O (8.7:1.3:1.7:0.4) for phenolics, and ethyl acetoacetate, toluene, and formic acid. Scanning was done at 254 and 366 nm using TLC scanner 4 (CAMAG) controlled by winCATS software after derivatization. Analysis of the mass spectra was performed with a TLC-MS interface (CAMAG) and ESI on a negative ion mode coupled quadrupole mass spectrometer. This technique allowed the identification of four compounds with their RF value and m/z value: theobromine (0.45, 181), caffeine (0.55, 195), phenolics (0.39, 353), saponins (0.16, 1219). A single HPTLC plate can be used to measure methylxanthines and phenolic chemicals, as well as to qualitatively evaluate saponins in mate tea [64].

Analysis of physalins in *Physalis alkekengi* through HPTLC-MS

Physalis alkekengi is one of the flowering plants of the family Solanaceae [65]. Bioactive secondary metabolites like physalins [66, 67], carotenoids [68], alkaloids, flavonoids [69], and other bioactive components were detected in the chemical analysis. Physalins have many pharmacological effects including antibacterial [70, 71], antitumor, anti-inflammatory, immunosuppressive, antinociceptive, antimalarial, leishmanicidal, anti-spermatogenic, and antifertility, which are still being studied. Physalins were examined in different parts of the *Physalis alkekengi* plant by Eva Kranjc et al. using HPTLC-MS. In a twin trough chamber, silica gel 60 HPTLC plates pre-developed with chloroform-methanol (1:1, v/v) and dried with developing solvent ethyl acetate – toluene – formic acid (7:3:0.2, v/v). Samples were applied using automated TLC applicator Linomat 5 (CAMAG) dipped into derivatizing reagent sulphuric acid, anisaldehyde, or molybdatophosphoric acid; TLC scanner 4 (CAMAG) controlled by winCATS software and coupled with triple quadrupole and ion trap mass analyzer detector was used for scanning at 245 and 366 nm. It was identified with improved sensitivity and certainty using this technique, the RF value of 0.60 was assigned to physalins with m/z value of 527 and the RF value of 0.53 to its impurity with m/z value of 529. By analysing fragmentation patterns in mass spectra, physalins and a common impurity were proven to be members of the same family. The impurity was identified as 2,3,25,27-tetrahydrophysalin A. HPTLC-MS of *Physalis alkekengi* L. can be used to obtain more informative and straightforward results, since it can be used to analyze physalins in different sections of the plant [72].

Identification of anti-microbial compounds from propolis using hyphenated HPTLC-MS

Propolis, a sticky mixture made up of plant exudates, is used by honeybees to protect their hive from infection [73]. Although propolis is often high in phenolic acids and flavonols, its chemical composition is complex and diverse. Due to their antibacterial, anti-inflammatory, antioxidant, immunomodulatory, antitumor, and anti-diabetic properties, they have traditionally been used as effective treatments for a wide range of disorders [74, 75]. HPTLC-MS analysis of propolis for the rapid detection of antimicrobial chemicals was completed by Deepak Kasote et al. The optimized chromatographic condition included silica gel pre-coated aluminium plates with automated TLC sampler 4 (CAMAG) with developing solvent methanol:water (60:40, v/v) in ADC2 development chamber and glass twin-trough chamber (CAMAG). Plates were dried through TLC plate heater III (CAMAG). WinCATS version software and a hyphenated TOF MS were used as detectors in the reprostar 3 documentation system to scan at 254 and 365 nm. Using this technique, pinocembrin with an RF value of 0.48 and a m/z value of 213 was identified as having antifungal activity against *Candida albicans*. Following that, three compounds namely pinobanksin, pinobanksin 3-o-pentanoate, and pinobanksin 2-methyl butyrate had RF values of 0.59, 0.39, and 0.21 for Gram-negative and Gram-positive bacteria, respectively, and m/z values of 271, 355, and 355 for Gram-positive bacteria. With an RF value of 0.89 and 179, caffeic acid showed anti-QS properties. Inhibitory experiments with violacein were used to validate it quantitatively. Because of its high sensitivity and selectivity, this method is ideal for rapidly detecting bioactive compounds in crude natural products. Moreover, HPTLC-MS proved to be an effective method for detecting bioactive chemicals in propolis within a short period of time [76].

Bio-profiling of *Salvia miltiorrhiza* using planar chromatography and HRMS

Salvia miltiorrhiza is used frequently as a folk medicine in Japan, the United States, and other western countries. The chemical analysis revealed presence of bioactive components, mainly phenolic acids, and tanshinones. Alzheimer's disease, Parkinson's disease, cerebrovascular illness, coronary heart disease, cancer, renal deficiency, bone loss, hepatocirrhosis, gynecologic diseases, chilblains, psoriasis, and carbuncles are among the therapeutic activities [77]. Ebrahim Azadniya et al. illustrated the HRMS with HPTLC for the bio-profiling of *Salvia miltiorrhiza*. HPTLC-MS chromatographic settings included HPTLC silica gel 60 F254 plates with developing solvent toluene-chloroform-ethyl acetate-methanol-formic acid (4:6:8:1:1) in ADC2 automatic development chamber employing automatic TLC sampler 4 (CAMAG). Scanning was done at 254 and 366 nm using TLC scanner 4 (CAMAG) and second development with petroleum ether – cyclohexane – ethyl acetate (5:2.8:2.2) as a developing solvent. Derivatization was carried out using the derivatizing agent anisaldehyde sulphuric acid reagent, which was immersed in a TLC immersion device (CAMAG) and heated with a TLC plate heater (CAMAG) with the reprostar 3 documentation system and winCATS version software. An interface between the TLC-MS system and a quadrupole-Orbitrap MS system and a heated ESI source. In this study, five acetylcholinesterase inhibitors with their m/z value like salvianolic acid B (717.14), lithiospermic acid (537.10), rosmarinic acid (359.07), cryptotanshinone (319.13), unidentified inhibitors were found in both 15,16-dihydrotanshinone I (304.10). Additionally, salvianolic acid, lithiospermic acid, and rosmarinic acid exhibit antioxidant activity, while cryptotanshinone and 15,16-dihydrotanshinone exhibit antibacterial activity. This method is effective for comprehensive bio-profiling of *Salvia miltiorrhiza* and screening of its extracts. This confirms its global acceptance as a natural product of great potential [78].

Analysis of fresh and dried *Sambucus nigra* (elderberry) using hyphenated planar chromatography

Sambucus nigra (elderberry) is a flowering plant species in the Adoxaceae family, and it is widely distributed throughout Europe, Asia, and North Africa [79]. Upon chemical analysis, we discovered anthocyanins, i.e., the flavonoids of plants found as glycosides, such as 3-O-glycosides or 3, 5-di-O-glycosides [80]. The chemical constituents from fruits are abundant in cyanidin derivatives such as

cyanidin-3-glucoside, cyanidin-3 sambubioside, and cyanidin-3-sambubioside-5-glucoside [81], flavanols such as quercetin and kaempferol glucosides, phenolic acids such as chlorogenic acid and its derivatives, procyanidins [82], and some trace amount of other anthocyanins. Additionally, there are terpenes and lectins, free and conjugated forms of amino acids, proteins, unsaturated fatty acids, fibre fractions, vitamins, and minerals in this extract. This plant has traditionally been used for diuretics and diaphoretics, as well as to treat common colds and influenza. In recent studies, researchers found that it is effective against viruses, diabetes mellitus, cardiovascular disease, and cancer. S. Kruger et al. used hyphenated planar chromatography with a single quadrupole MS to examine the effects of fresh and dried elderberry. HPTLC silica gel 60 F254 plates with automatic TLC sampler 4 (CAMAG) with developing solvents ethyl acetate – 2-butanone – formic acid – water (5:3:2:1) in automatic development chamber were used to optimize chromatographic conditions for HPTLC-MS (CAMAG), scanning was done between 200–700 nm via TLC scanner 4 (CAMAG), derivatization with anisaldehyde sulphuric acid reagent, immersion in TLC immersion device (CAMAG), heating with TLC plate heater (CAMAG) with reprostar 3 documentation system, data processing with winCATS software, and hyphenation with single quadrupole mass spectrometer was incorporated in this instrument. In this study, Electrospray ionization mass spectrometry (HPTLC-ESI + /ESI − MS) was used to analyze cyanidin-3-sambubioside and cyanidin-3-glucoside, and other bioactive zones, with m/z values of (581 and 449), leading to discovery of multipotent compounds. It is a method that can be used for quantitation, effect-directed analysis, bio-profiling, pattern recognition, and fingerprinting of elderberry plants, in addition to their potential herbal analysis [83].

Identification and quantification of bioactive components of milk thistle using HPTLC-MS.

Since the first century, *Silybum marianum* has been one of the most commonly utilized plant species in Europe [84]. The chemical constituent isolated from *Silybum marianum* rich in flavonolignans, namely silymarin, silibinin, iso silibinin, silicristin, and silidianinin [85]. Milk thistle has been shown in recent studies to have anti-inflammatory, anticarcinogenic, hypocholesterolemic, immunomodulating, congestion, and menstrual irregularities effects [70]. Mahmoud N. Taha et al. used single-quadrupole MS for HPTLC to identify and quantify milk thistle bioactive components. HPTLC plates silica gel 60 F254 were used with the automatic TLC sampler and the solvent system toluene – ethyl acetate – formic acid (9:6:0.4 v/v/v) in the twin trough chamber (CAMAG) at a temperature of 25 ˚C for HPTLC-MS analysis. TLC visualizer (CAMAG) was used to document the chromatograms, which were recorded using the TLC scanner 3 and winCATS software between 200 and 700 nm wavelength (CAMAG). Using the TLC-MS Interface (CAMAG) in conjunction with the ESI interface of the single-quadrupole mass spectrometer, mass spectra were obtained. The mobile phase method, with hRF values of 36 for silibinin, allowed for satisfactory resolution of the marker chemicals and validation of bioactive chemicals by HPTLC-MS by recording mass spectra. In negative ion mode, silibinin in milk thistle had an m/z ratio of 481, accompanied by a small fragment of 301 [F]-. For the analysis of natural products, hyphenated HPTLC is recommended since it is a powerful research method that can measure and detect silibinin, a phytochemical found in *Silybum marianum* [35].

Quantification of bioactive components in ginger (*Zingiber officinale*) using HPTLC-MS

Since ancient times, *Zingiber officinale*, a sultry plant has been used as a food condiment. The rhizomes of fresh ginger are often used as spices, dietary supplements, and traditional medicine. Among its volatile compounds are essential oils, non-volatile oleoresins, phenols, gingerols, shogaols, and fixed oils, all liable for giving it its scent, flavor, and pungent taste [86]. Antioxidant, anti-inflammatory, antimicrobial, antifungal, and acetylcholinesterase inhibitory [87] are some of its beneficial medicinal properties [88–90]. Pharmacologically, it is beneficial in reducing blood pressure, enhancing blood coagulation, reducing inflammation, and helping the gastrointestinal tract [91]. It also has antioxidative properties in vitro and in vivo. HPTLC with a hybrid quadrupole-Orbitrap MS was used to quantify bioactive substances by Ginger S. Kruger et colleagues. Optimized chromatographic conditions for HPTLC-MS were designed in a twin trough glass chamber (CAMAG) using a silica gel 60 F254 precoated HPTLC plate with automatic TLC sampler 4 (CAMAG) and a solvent system of hexane and ethyl acetate (13:7). Anisaldehyde sulphuric acid reagent and primuline reagent were used to derivatize the plate, which was heated to 110 ˚C using a TLC plate heater (CAMAG). Scanning was done at 540 nm, processed through winCATS software via mass detector single quadrupole incorporated in the instrument. These results suggest that the content of 6-gingerol and shogaol in ginger is between 0.2–7.4 mg/g and 0.2–3.0 mg/g, respectively, and that their m/z values are (317.17 and 349.24). HPTLC-ESI-MS characterized the discovered unknown bioactive zones assigned as 8 and 10 gingerols. In conclusion, it is a highly sensitive and precise method for profiling the bioactive constituents of ginger, and it informs about the food chain and quality control of products [92].

Conclusion

Over 80% of the world's population uses traditional plant-based medicine for providing basic medical care, according to the World Health Organization. According to estimates, the worldwide herbal medicine market will be worth USD 411.2 billion by 2026. In the recent years, it has found tremendous application in several areas such as the analysis of herbal drugs, the quality control, and the standardization of herbal drugs. HPTLC-MS has several advantages over standard chromatographic procedures. The present study focuses on resolving quality issues in herbal products. The primary concern is a lack of standardization, identifying active constituents which matter to the quality of herbal products. Moreover, in this study, the RF values and mass to charge ratio of 15 herbal products were analyzed using all the chromatographic conditions with MS applied. An analysis of HPTLC's recent applications in the analysis of herbal drugs along with hyphenated techniques has also been described in this paper. As a result of this assembling, researchers will gain insight into HPTLC-MS techniques that can be used to resolve herbal drugs' quality control problems based on their fingerprint spectra. The phytochemical composition of a plant extract or formulation is represented by its fingerprint, also, it can be used for monitoring batch to batch consistency and stability tests of herbal medicines and dietary supplements.

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