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A Review on Phytoconstituent Analysis by Different Analytical Techniques

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ABSTRACT

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads. Due to an increasing demand for seeking therapeutic drugs from natural products there is a need to develop analytical methodologies for different purposes which include the extraction, isolation and characterization of active ingredients in botanicals and herbal preparations. Various analytical methods available for the isolation, characterization and quantification of active ingredients from plant extracts which include various spectrometric and chromatographic methods along with some hyphenated techniques are discussed. The present review helps to design a method for the isolation, characterization and quantitative determination of major constituents from plants.

Keywords: Natural products, isolation, characterization, quantification, analytical techniques.

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INTRODUCTION

Medicinal plants have played a key role in world health. Inspite of great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. According to World health organization (WHO) 65-80% of the world's population living in developing countries depends especially on plants for primary health care[1]. Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological activities, higher safety margins and lesser costs [2]. Therefore, analysis of major components in herbs is of importance for research and quality evaluation of these botanical drugs. Especially a comparative analysis on herbal drugs collected from different origins or different medicinal portions was very useful for choosing the best origin or medicinal portion [3]. Selection of chemical markers is crucial for the quality control of herbal medicines, including authentication of genuine species, harvesting the best quality raw materials, evaluation of post harvesting handling, assessment of intermediates and finished products, and detection of harmful or toxic ingredients. Ideal chemical markers should be the therapeutic components of herbal medicines. However, for most herbal medicines, the therapeutic components have not been fully elucidated or easily monitored. Bioactive, characteristic, main, synergistic, correlative, toxic and general components may be selected [4]. Hence a thorough validation of herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phyto-chemicals have complementary and overlapping mechanism of action [5]. Moreover, adulteration and contaminations are regular events in herbal drug manufacturing. To ensure that raw materials used in manufacturing of drugs are not only authentic but also of prescribed quality, identification and evaluation of raw materials has become fundamental need of herbal industry. Thus fingerprinting and marker compound analysis by chemical and validated chromatographic techniques are gaining importance for use in standardizing herbal medicinal formulations[6]. Chromatography and spectroscopy techniques are most commonly used methods in standardization of herbal medicines but the herbal system is not easy to analyse because of their complexity of chemical composition. Chromatography plays a fundamental role as an analytical technique for quality control and standardization of phytotherapeuticals[7]. World health organisation has also accepted chromatographic fingerprint technique for the evaluation of the quality of the herbal medicines. The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation. A summary of general approaches in extraction, isolation and characterization of bioactive compound from plant extract is shown in figure 1[8].

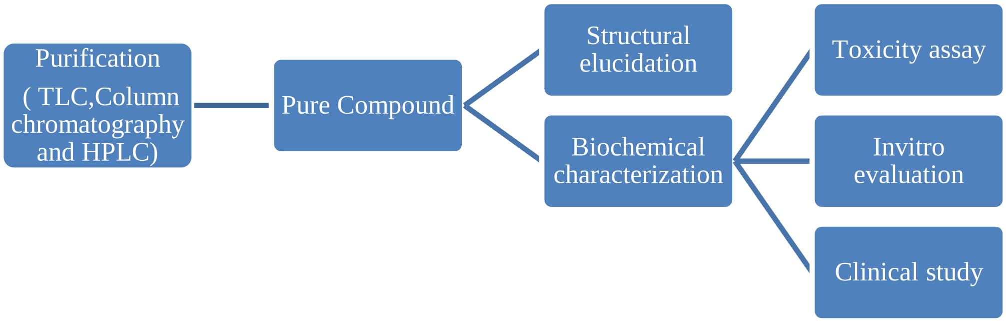


Figure 1 A brief summary of the general approaches in extraction, isolation and characterization of bioactive compound from plants extract

The following provides a brief review of different analytical methods available for phytoconstituent analysis.

There are different steps involved in analysis of phytoconstituents from plants which include:

a. Extraction (sonification, heating under reflux, soxhlet extraction and hyphenated techniques like super critical fluid extraction, microwave assisted extraction are used for the extraction purposes).

b. Isolation of phytoconstituents from the plant extract by using different techniques like column chromatography, preparative chromatography, sublimation, distillation, fractional crystallization.

c. Characterization of the isolated compounds

Due to the fact that plants extracts usually occur as a combination of various types of bioactive compounds or phytocompounds with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. Once the constituent is isolated from the extract then characterization is done using methods like melting point, UV-Visible, fourier transform-infrared, 1H NMR, 13 C nuclear magnetic resonance and mass spectrometric studies. Characterization by different analytical methods provides the accurate identity of the isolated phytoconstituent.

d. Quantification by different analytical methods

Quantification of the separated constituents is done by using different methods like,

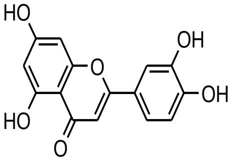
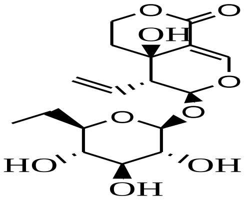
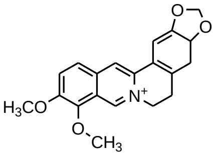
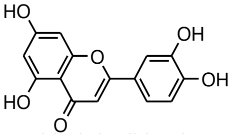
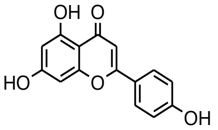
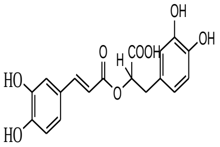
High performance thin layer chromatography (HPTLC), high pressure liquid chromatography(HPLC), combination of HPLC and HPTLC methods, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, ultra high performance liquid chromatography-mass spectrometry, nuclear magnetic resonance spectroscopy (NMR) methods and combination of high performance liquid chromatography, high performance thin layer chromatography, gas chromatography-mass spectrometry, and combination of nuclear magnetic resonance and mass spectrometric methods.

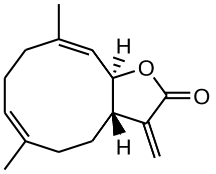
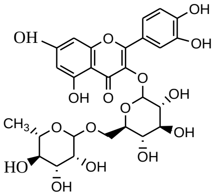
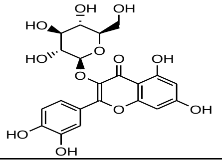
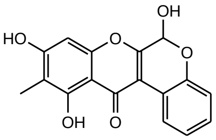
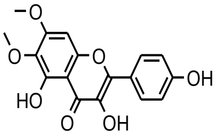
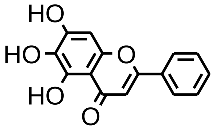
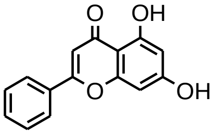
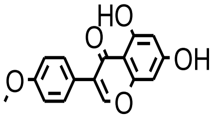
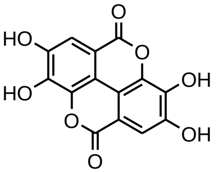
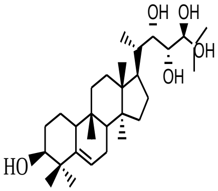
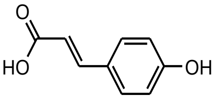
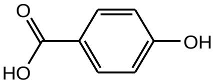
e. After developing a method for quantitative estimation of phytoconstituents from plants, the developed method is validated for different parameters like accuracy, precision, linearity, limit of detection and limit of quantitation [9].

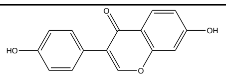
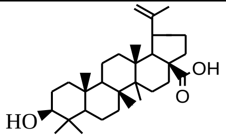
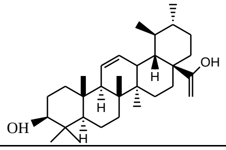
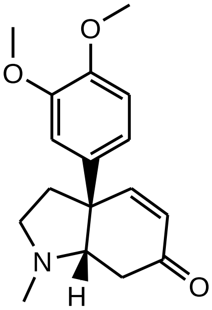
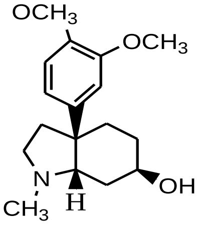
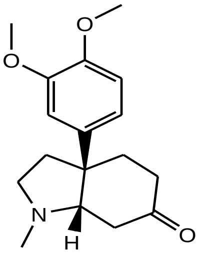
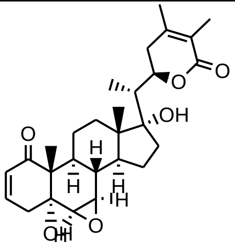
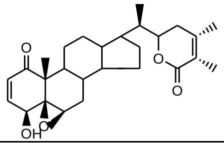
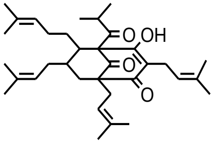
Many Validated Chromatographic Methods are Used for the Quantification of Phytoconstituents from Plants. Some of them Include

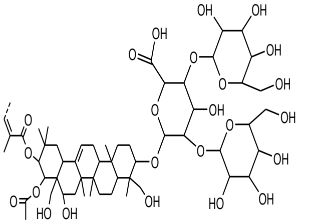
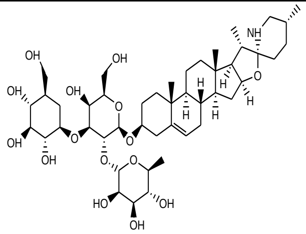
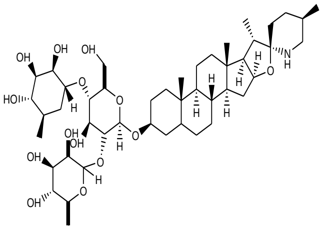
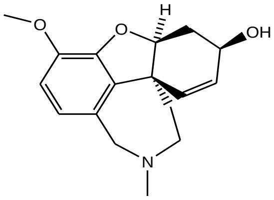
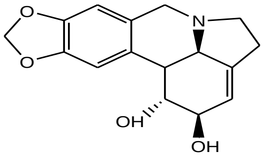
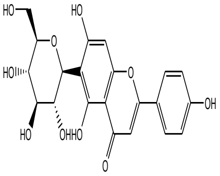
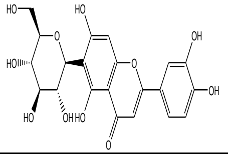
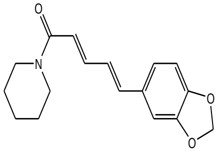
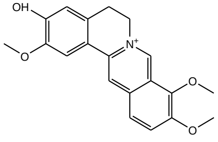
A summary of different plant constituents isolated and quantified from plant extracts along with the analytical methods used is shown in table 1.

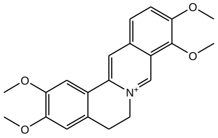
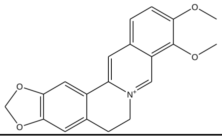
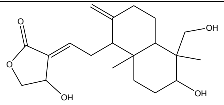
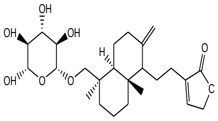
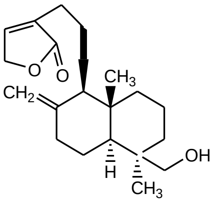
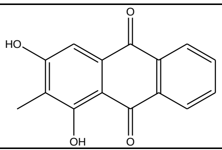
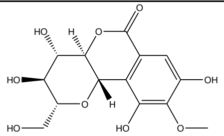
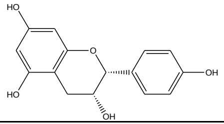
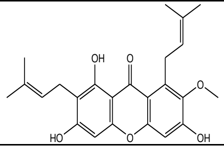
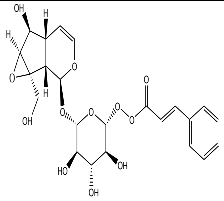
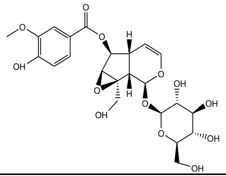
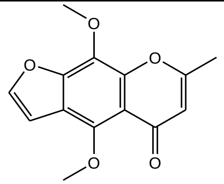
Table 1: Table showing constituents quantified by different analytical methods

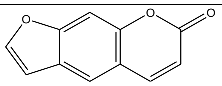
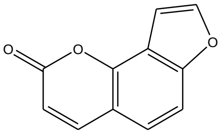
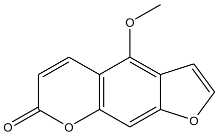
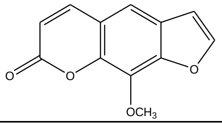
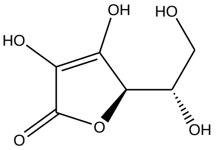
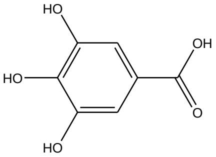
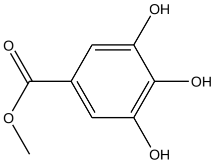
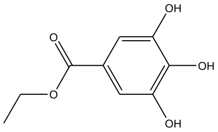
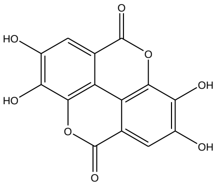
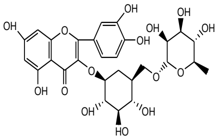
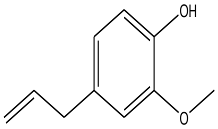
S.No	**Plant Name**	**Constituent**	**Chemical structure**	**Analytical method**	**References**
1.	*Vitex negundo*	flavone type of flavonoid-luteolin	 2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone	HPLC	10
2.	*Enicostemma littorale*	secoiridoid glycoside-swertiamarin		HPLC-UV	11
3.	*Berberis aristata, Berberis tinctoria*	Protoberberine group of isoquinoline alkaloid-Berberine		HPLC	12
4.	*Achillea millefolium*	Flavonoid-Luteolin, Flavone- Apigenin	 2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone 	RP-HPLC	13
5.	*Thunbergia laurifolia*	Caffeic ester-Rosamarinic acid		HPLC	14

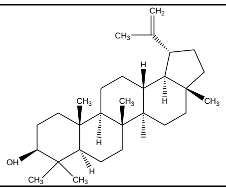
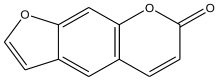
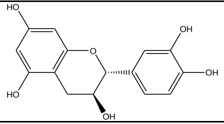
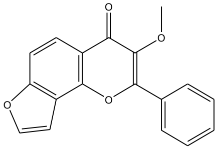
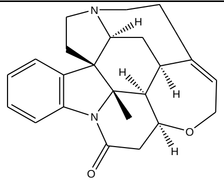
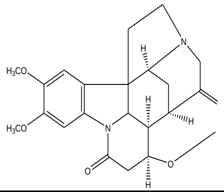
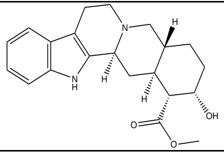
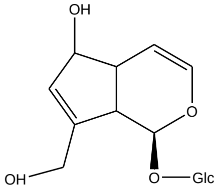
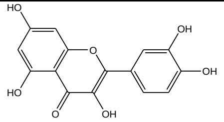
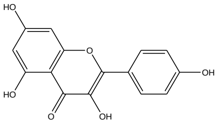
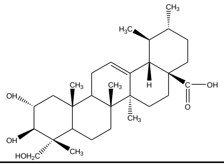
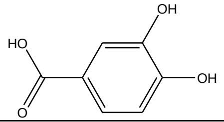
6.	*Saussurea lappa*	Sesquiterpene lactone-Costunolide		HPLC	1
7.	*Jasminum sambacait*	Flavonoid glycoside-Rutin, quercetin glycoside-Isoquercetin	 	RP-HPLC	15
8.	*Boerhavia diffusa*	Rotenoid-Boerhavinone B, Glycoside-Eupalitin-3-O-β-D-galactopyranoside	 	HPLC	16
9.	*Oroxylum indicum*	Flavone type flavonoids-Baicalein and chrysin, O-methylated flavone-Biochanin-A, Natural phenol-Ellagic acid	   	RP-HPLC	17
10.	*Momordica charantia*	Curcubitane triterpenoids-momordicosides		HPLC	18
11.	*Macrotyloma uniflorum*	Phenolic acids: p-coumaric acid, p-hydroxy benzoic acid	 	RP-HPLC	19

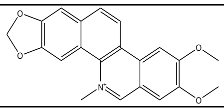
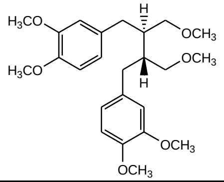
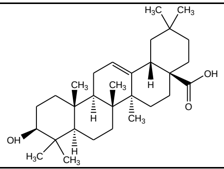
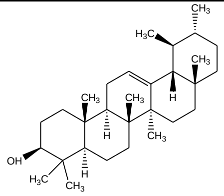
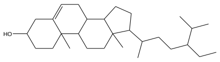
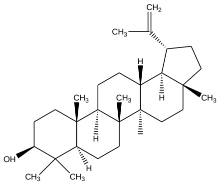
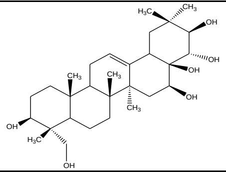
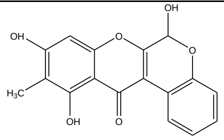
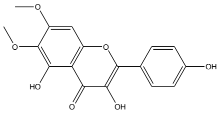
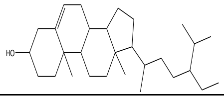
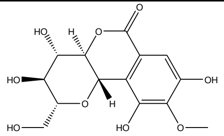
12.	*Momordica dioica*	Isoflavone-daidzein		HPLC	20
13.	*Mimusops elengi*	Pentacyclic triterpenoids-Betulinic acid, ursolic acid		HPLC	21
14.	*Sceletium* plant material	Mesembrine type alkaloids-mesembrenone, mesembranol, mesembrine		HPLC	22
15.	*Withania somnifera*	Withanolides-Withanone, Withaferine-A		HPLC	23
16.	*Hypericum perforatum*	Prenylated phloroglucinol derivative-Hyperforin		HPLC	24

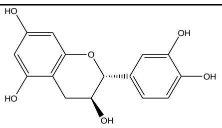
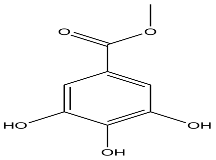
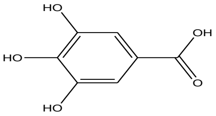
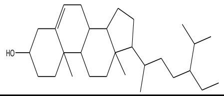
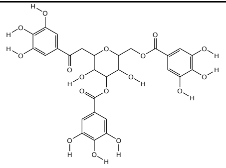
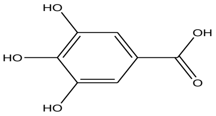
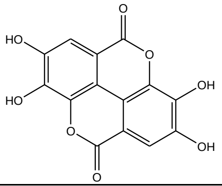
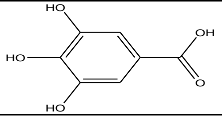
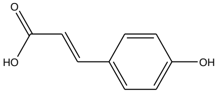
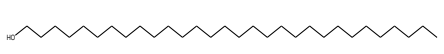
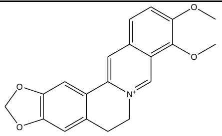
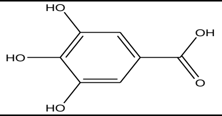
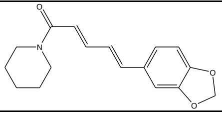
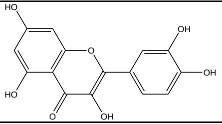
17.	*Aesculus hippocastanum*	Triterpenoid saponin-B-Escin		HPLC	25
18.	*Solanum lycocarpum*	Glycoside-Solasonine, Glycoalkaloid derivative-solamargine	 	HPLC	26
19.	*Galanthus elwesii*	Alkaloids-Galanthamine, Lycorine	 	HPLC-DAD	27
20.	*Cecropia* species	Flavones-Isovitexin, Iso-orientin	 	HPLC-DAD	28
21.	*Piper nigrum*	Alkaloid-Piperine		HPLC	29
22.	*Tinospora cordifolia*	Protoberberine alkaloids-Jatrorrhizine and Palmatine, protoberberine group of		RP-LC-DAD	30

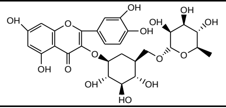
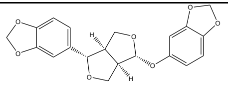
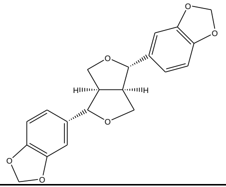
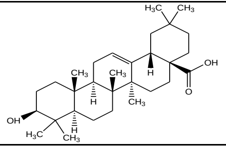
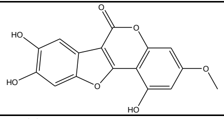
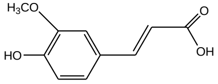
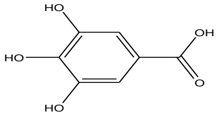
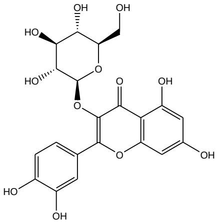
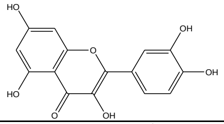
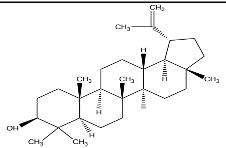
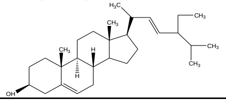
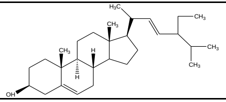
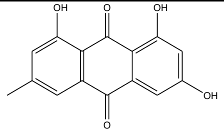
		isoquinoline alkaloid-Berberine	 		
23.	*Andrographis paniculata*	Labdane diterpenoid-Andrographolide, Diterpene glucoside-neo-andrographolide, Diterpene lactone-Andrograpanin	  	HPLC	31
24.	*Rubia cordifolia*	Anthroquinone-Rubiadin		RP-HPLC	32
25.	*Bergenia ligulata* Yeo	Trihydroxy benzoic acid glycoside-Bergenin, flavan-3-ol type flavonoid-Afzelechin	 	HPLC	33
26.	*Garcinia mangostana* Linn	Natural xanthonoid-α mangostin		HPLC	34
27.	*Picrorhiza kurroa*	Iridoid glycosides-Picroside-I, picroside-II	 	HPLC	35
28.	*Ammi visnaga* Lam	Furanochromone-Khellin		RP-HPLC	36

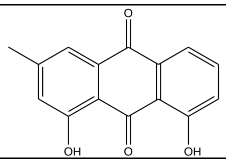
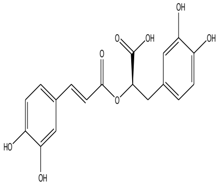
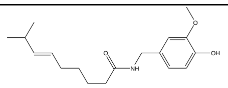
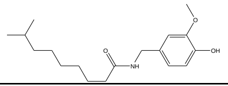
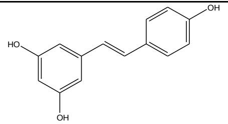
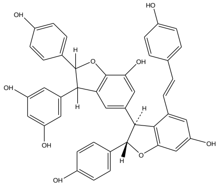
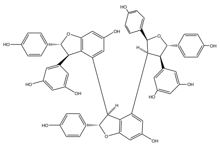
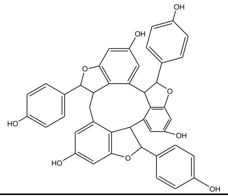
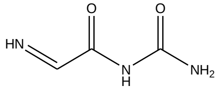
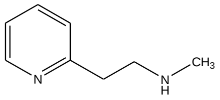
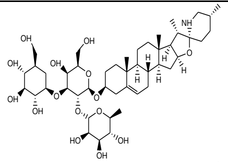
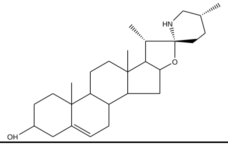
29.	*Psoralea Coryfolia, Ammi majus*	Furanocoumarins-Psoralen, Angelicin, 5-methoxy psoralen, 8-methoxy psoralen	   	HPLC	37
30.	*Phyllanthus emblica*	Phenolic acids-Ascorbic acid, Gallic acid, phenolic compound-Methylgallate, ester of caffeic acid and quinic acid-Chlorogenic acid, ethyl ester of gallic acid- Ethyl gallate, Natural phenol antioxidant-Ellagic acid, flavonoid glycoside-Rutin	     	HPLC	38
31.	*Cinnamomum zeylanicum, Cinnamomum tamala, Myristica fragrans, Ocimum sanctum*	Phenyl propanoid-Eugenol		HPLC	39

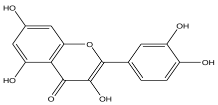
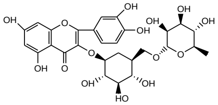
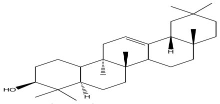
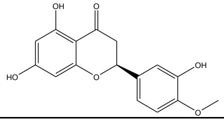
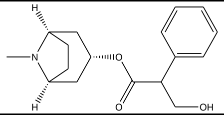
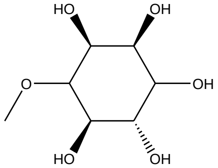
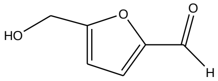
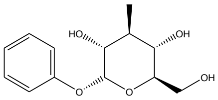
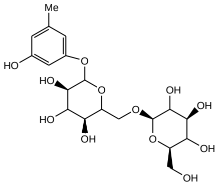
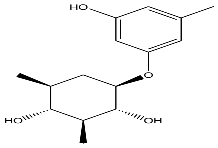
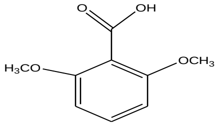
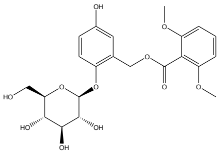
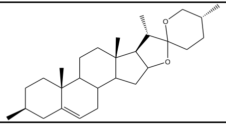
32.	*Strobilanthes ciliates* Nees	Triterpenoid-Lupeol		HPTLC	40
33.	*Psoralea corylifolia*	Furanocoumarin-Psoralen		HPTLC	41
34.	*Anacardium occidentale*	Natural phenol-Catechin		HPTLC	42
35.	*Pongamia pinnata*	Flavonol type of flavonoid-Karanjin		HPTLC	43
36.	*Strychnos Nux-vomica*	Alkaloid-Strychnine, Brucine	 	HPTLC	44
37.	*Rauvolfia tetraphylla*	Indole alkaloid-Yohimbine		HPTLC	45
38.	*Vitex-agnus castus L.*	Iridoid glycoside-Aucubin		HPTLC	46
39.	*Centella asiatica*	Flavonol-Quercetin, flavonol type flavonoid-Kaempferol, Triterpene-Asiatic acid	  	HPTLC	47
40.	*Amumum subulatum* Roxb	Phenolic acid-Protocatechuic acid		HPTLC	48

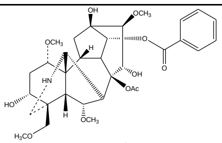
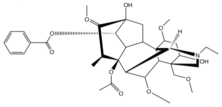
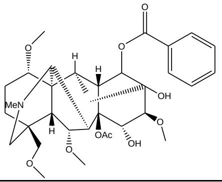
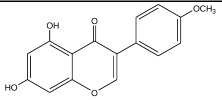
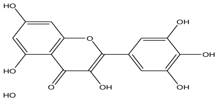
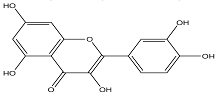
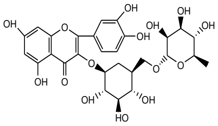
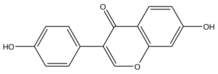
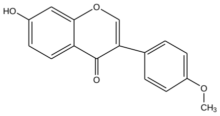
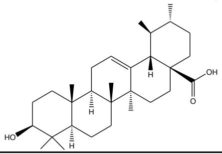
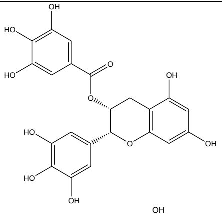
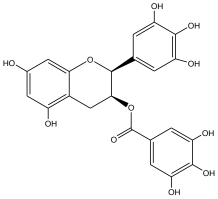
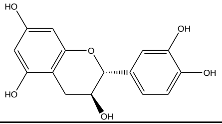
41.	*Toddalia asiatica* Linn	Benzophenanthridine alkaloid-Nitidine		HPTLC	49
42.	*Phyllanthus amarus*	Bioactive lignan component-Phyllanthin		HPTLC	50
43.	*Satureja mutica* fisch	Triterpenoid-Oleanolic acid		HPTLC	51
44.	*Leptadenia reticulata*, *Pluchea Lanceolata*	Triterpene-α amyrin, phytosterol-β-sitosterol, triterpenoid-Lupeol, higher alkane-n-triacontane	   	HPTLC	52
45.	*Gymnema sylvestre*	Triterpenoid glycoside-Gymnemagenin		HPTLC	53
46.	*Boerhavia diffusa*	Roenoid-Boerhavinone-B, glycoside-Eupalitin-3-O-β-D-galactopyranoside, phytosterol-B-sitosterol	  	HPTLC	54
47.	*Bergenia ciliata*	Trihydroxy benzoic acid glycoside-Bergenin, Natural phenol-Catechin,		HPTLC	55

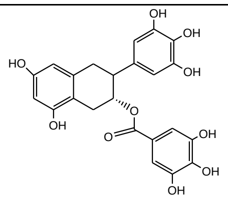
		Methyl ester of gallic acid-Gallicin, Phenolic acid-Gallic acid, Phytosterol-β-sitosterol	   		
48.	*Terminalia chebula*	Polyphenol-Tannic acid, Phenolic acid-Gallic acid, Natural phenol antioxidant-Ellagic acid	  	HPTLC	56
49.	*Diospyrus Ferrea,* *Aerva lanata*	Phenolic acid-Gallic acid		HPTLC	57
50.	*Stereospermum suaveolens* Roxb.	Phenolic acid-p-coumaric acid, fatty acid-triacontanol	 	HPTLC	58
51.	*Berberis asiatica*	Protoberberine group of isoquinoline alkaloid-Berberine		HPTLC	59
52.	*Careya arborea*	Phenolic acid-Gallic acid		HPTLC	60
53.	*Piper nigrum* *Piper longum*	Alkaloid-Piperine		HPTLC	61
54.	*Saraca asoca*	Flavonol-Quercetin Flavonoid glycoside-Rutin		HPTLC	62

					
55.	*Sesamum indicum*	Lignans-Sesamolin, Sesamin	 	HPTLC	63
56.	*Acyranthes aspera* Linn	Triterpenoid-Oleanolic acid		HPTLC	64
57.	*Eclipta alba*	Naturally occurring coumestan-Wedelolactone		HPTLC	65
58.	*Boerhavia diffusa*	Flavonoids-Ferulic acid, Gallic acid, isoquercetin, quercetin	   	HPTLC	66
59.	*Abutilon indicum*	Triterpenoid-Lupeol, phytosterol-Stigmasterol	 	HPTLC	67
60.	*Acalypha indica*	Phytosterol-Stigmasterol		HPTLC, HPLC	68
61.	*Rheum emodi*	Trihydroxy anthroquinone-Emodin, anthroquinone-Chrysophanic acid		HPLC, HPTLC	69

					
62.	*Thymus x citriodorus*	Phenolic compounds-Caffeic ester-Rosamarinic acid, flavones-Luteolin-7-O-αglucuronide		HPLC-UV-ESI-MS	70
63.	*Capsicum* fruits	Capsaicinoids-Capsaicin, Dihydrocapsaicin	 	LC-TOF-MS	71
64.	*Carex* Species	Glycoside-Resveratrol-diglucoside, stilbenoids-miyabenol A and C, Kobophenol A stilbene trimer-Alpha-viniferin	   	HPLC-MS	72
65.	*Acalypha alnifolia*	Phenolic compounds-Cyanoacetyl urea, 4-(2-methyl amino) ethyl pyridine (betahistidine)	 	GC-MS	73
66.	*Solanum nigrum*	Glycoside-Solasonine, Alkaloid-Solasodine	 	HPLC, GC-MS	74

67.	*Delonix elata* (L.) gamble	Flavonol-Quercetin, Flavonoid glycoside- Rutin Pentacyclic triterpenoid-Beta amyrin, Citrus bioflavonoid-Hesperitin	   	GC-MS	75
68.	*Hyoscyamus reticulata*	Tropane alkaloid-L-hyoscyamine		GC-MS	76
69.	*Argyrolobium roseum*	Cyclic polyol-Pinitol		1H-NMR	7
70.	*Curculigo orchioides*	Beta-hydroxyl methyl furfural, phenyl-β-D-glucopyranoside, phenolic glucosides-Anacardoside, Orcinol glucoside, Orcinol-1-O-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside, 2,6 dimethoxy benzoic acid, curculigoside, curculigine	     	UPLC-ESI-Q-TOF/MS	77
71.	*Dioscorea* species	Steroid sapogenin-Diosgenin		UPLC-PAD-MS	78

72.	*Aconitum* herbs	Aconitum alkaloids-Mesaconitine, Aconitine, Hypaconitine, Benzoyl mesaconitine, Benzoyl hypaconitine	  	UPLC-MS	79
73.	*Ixora coccinea*	O-methylated isoflavone-Biochanin-A, flavonoid class of polyphenolic compound-Myricetin, flavonol-Quercetin, Flavonoid glycoside-Rutin, Isoflavone-Daidzein, O-methylated isoflavone-Formononetin, pentacyclic triterpenoid-ursolic acid	      	HPLC, HPTLC, GC-MS	80
74.	*Camellia sinensis*	Catechins-Epigallocatechin-3-O-gallate, Epigallocatechin, Epicatechin-3-O-gallate,Catechin, Gallocatechin, Gallocatechin-3-O-gallate	  	qNMR, LC-MS/MS	81



High performance liquid chromatography methods

A high performance liquid chromatographic method is developed by Lubna et al., for the assessment of extraction methods and quantitative estimation of luteolin from the leaves of *Vitex negundo* Linn. Extraction is done by different techniques like maceration, reflux, soxhlet and ultrasound assisted extraction using four different solvents of varying polarity such as methanol, ethanol, chloroform and dichloromethane. High performance liquid chromatographic analysis to quantify luteolin from the extract was done using lichrosphere C-18 reverse phase column with mobile phase composed of methanol and 1% aqueous acetic acid solution (99:1 v/v) at a flow rate of 1ml/min. The calibration plot of standard luteolin showed a linear relationship in the concentration range of 100-500µg/ml with a correlation coefficient of 0.998. The methanolic extract was found to contain highest amount of luteolin and for the extraction and isolation reflux technique was observed to be most efficient[10]. A validated high performance liquid chromatographic method is developed for the estimation of swertiamarin in *Enicostemma littorale* by Javed et al., Extraction (whole plant) is done using methanol and water (80:20 v/v) as solvent with the help of ultra-sonicator. The developed high performance liquid chromatographic method with UV detection was found to be accurate and reproducible. A well-resolved peak of swertiamarin appeared at Rt 3.51±0.07 min with methanol-water (80:20) as mobile phase. The linear regression analysis data for the calibration curve showed good linearity in the concentration range of 100-1000 µg/ml with a correlation coefficient (r2) of 0.9994. The limit of detection and limit of quantitation were found to be about 19 and 58µg/ml, respectively. The method was found to be accurate (98.09-100.70 % recovery) and precise (< 2 % R.S.1.5 % RSD)[11]. A reverse phase high performance thin layer chromatographic method with photodiode array detection has been developed by Hemant et al., for the determination of major constituent berberine from *Berberis aristata* and *Berberis tinctoria*. Berberine was isolated from the plant extract on semi-preparative high performance liquid chromatography and separated on high performance liquid chromatography by using isocratic mode using mobile phase consisting of 0.1% trifluoroacetic acid: acetonitrile (60:40, v/v) at a flow rate of 1mL/min. Structure of the isolated constituent was confirmed by melting point, high performance thin layer chromatography and spectral analysis like nuclear magnetic resonance, infrared and mass spectrometry. The purity of berberine was found to be more than 99.8%. A molecular ion peak corresponding to molecular weight of berberine is observed (336). A plot of integrated peak area versus concentration of berberine was found be linear over the concentration range of 0.2µg/mL to 150µg/mL. The limit of detection was 1ng on column and limit of quantification was 2 ng on column for berberine. The berberine content in *B. aristata* and *B. tinctoria* was found to be 3.18% and 1.46% respectively[12]. Andriy have developed a high performance liquid chromatographic method for the simultaneous determination of luteolin and apigenin (flavonoids) in herb of *Achillea millefolium* L. Luteolin and apigenin were used as calibration standards. The analysis was performed using a Waters XTerra C18 column (250 x 4.6 mm i.d., 5 µm particle size), as stationary phase, with a flow rate of 1 mL/min and detection at a wavelength of 350 nm. The proposed method was validated according to ICH guidelines. An excellent linearity was obtained with r values higher than 0.99. Besides, the chromatographic peaks showed good resolution. With other validation data, including precision, specificity, accuracy and robustness, this method demonstrated good reliability and sensitivity[13]. A HPLC method is developed by Pipob et al., for anti-oxidant guided isolation of rosamarinic acid, a major constituent from *Thunbergia laurifolia*. Extraction is done using ethanol as solvent by maceration technique. Isolation of rosamarinic acid is done based on its antioxidant activity. The characterization of isolated rosamarinic acid is done by using UV, NMR and mass analysis. The high-performance liquid chromatography (HPLC) quantification of rosmarinic acid was accomplished using C-18 Cosmosil column and water: methanol: acetic acid (65:35:0.1) as mobile phase. The developed method was applied to determine the amount of rosmarinic acid in *T. laurifolia* samples. The HPLC method was also validated in terms of linearity, precision, and accuracy. Rosmarinic acid content in *T. laurifolia* varied from 1.56 to 53.32 mg/g of dry plant[14]. The determination of costunolide as marker of *Saussurea lappa* and its herbal formulations is done by Nageswara et al., using HPLC technique. Powdered roots were extracted using different solvents like hexane, ethyl acetate, chloroform and methanol with the help of sonicator. Isolation of costunolide from the extract was done by column chromatography and characterization is done by NMR and mass spectral data. Chromatographic separation is done using acetonitrile and water (60:40 v/v) as mobile phase at flow rate of 1.0mL/min. The method was validated for linearity, precision, accuracy, and system suitability. The average recovery of the method at three levels was 92.16-100.84% and linearity was good for costunolide over a relatively wide range of concentration (1.0-100 µg/mL). The costunolide content in the extract and different formulations varied from 0.66±0.01 to 11.29±0.34 mg/100g [1]. A HPLC method for the determination of rutin and isoquercitrin from the leaves of *Jasminum sambacait* is developed by Vidhya and Dhanashri. Finely powdered leaves of *Jasminum sambacait* were extracted using methanol as solvent with the help of a sonicator. The chromatographic separation was performed on a Phenomenex C18 column (250 x 4.6 mm, i.d. 5 µm) with mobile phase, comprising 0.2 % tri fluoro acetic acid in distilled water and acetonitrile (75:25 v/v). The detector response was linear for concentrations ranging from 0.1µg/mL to 100.0µg/mL and 0.050µg/mL to 250.0µg/mL for rutin and isoquercitrin (correlation coefficient=0.999). The developed method is found to be precise and the amounts of rutin and isoquercitrin in the dried leaf powder of *Jasminum sambaca*it were found to be 0.4959 mg/g and 0.6481mg/g respectively. The values of percent recovery were found to be 98.62% and 98.80% for rutin and isoquercitrin respectively [15]. Vaidya et al., has developed a HPLC method for the analysis of boerhavinone-B and eupaliptin-3-O-β-D-galactopyranoside from plant and formulation of *Boerhavia diffusa* Linn. Extraction (of whole plant powder) is done using soxhlet apparatus and methanol as solvent. Chromatographic separation is done using a gradient mixture of acetonitrile and water at a flow rate of 1.0ml/min and detection wavelength of 270nm. The developed HPLC method was validated for different parameters. Validation of the method showed response was a linear function of concentration in the range 10–120 µg mL−1 for Boeravinone B and 5–60 µg mL−1 for Eupalitin-3-O-β-D-galactopyranoside. The method was suitably validated and was found to be precise and robust. The assay value for plant sample of *B.diffusa* was found to be 0.43% and 0.1% for boeravinone-B and eupalitin-3-O-β-D-galactopyranoside [16]. Maitreyi et al. has developed a HPLC method for the quantification of baicalein, chrysin, biochin-A, ellagic acid from root bark of *Oroxylum indicum*. Powdered root bark after defatting with petroleum ether is successively extracted using different solvents like chloroform, ethyl acetate and n-butanol. Quantification was performed using reverse phase high performance liquid chromatography (RP-HPLC) fingerprint with water, methanol, acetonitrile and orthophosphoric acid as the mobile phase and detection was performed at 262 nm. Standard baicalein, chrysin biochanin-A and ellagic acid (Sigma-Aldrich) were employed for the development of the method. The method was precise with relative standard deviation for these constituents that ranged between 0.5-1.0% (interday). The developed method was found to be accurate, precise, rapid, simple, and selective for quantitative monitoring of chrysin, baicalein, ellagic acid and biochanin-A in the root bark of *Oroxylum indicum*. The hydrolysed *n*-butanol fraction showed 9.86 % of baicalein as compared to 3.24% in the petroleum ether fraction [17]. A HPLC method is developed by Yanhong et al., for the determination and quantitation of five curcubitane triterpenoids in Momordica charantia by RP-HPLC and evaporative light scattering detection. Extraction is done using methanol as solvent with the help of a sonicator. The triterpenoids present in the fruits of *Momordica charantia* are separated with an acetonitrile (0.1% acetic acid)–water (0.1% acetic acid)–methanol (0.1% acetic acid) gradient at a flow rate of 0.5 mL/min. The method was validated for precision, repeatability, and accuracy. The relative standard deviation was between 0.6–4.4%. The method was sensitive, quick, and accurate for the determination of main triterpenes and saponins in *Momordica charantia*. The content of triterpenoids found is 0.234, 0.152, 0.086, 0.110 and 0.035mg/100mg of dry plant material weight respectively [18]. Kawsar et al., has developed a RP-HPLC method for the identification and quantification of phenolic acids in *Macrotyloma uniflorum*. Extraction is done using ethanol as solvent and further isolation of phenolics from the extract is done. Eight phenolic acids namely, 3,4-dihydroxy benzoic, vanillic, caffeic, p-coumeric, ferulic, syringic and sinapic acids were isolated from an ethanolic extract of Macrotyloma uniflorum. The most abundant phenolic acids were p-coumaric acid and p-hydroxy benzoic acid [19]. Kale and Laddha developed a HPLC method for the isolation, characterization and quantification of isoflavone in Momordica dioica Roxb. Ex wild (curcubitaceae) fruits. Isoflavone daidzein was isolated from the fruits of *M. dioica* by soxhlet extraction followed by column chromatography. The isolated compound was identified by UV visible spectroscopy, IR spectroscopy, Mass spectrum, 1H-NMR and 13C-NMR, TLC and chemical test. HPLC analysis is done using an isocratic mobile phase, methanol and water containing 0.1% ortho-phosphoric acid (60:40 v/v) at a flow rate of 1.0 ml/min. The wavelength of maximum absorption (λmax) selected for quantification of daidzein was 255 nm. Calibration plot for daidzein was found to be linear with correlation coefficient (r2 = 0.9997) and regression equation ($y = 60805x - 77107$). The amount of daidzein present in *M. dioica* fruits was found to be 0.03% w/w [20]. A High pressure liquid chromatographic method is developed by Vidhya and Dhanashri for the simultaneous quantitation of betulinic acid and ursolic acid (triterpenic acids) from dried stem bark powder of *Mimusops elengi* Linn. Extraction is done using methanol as solvent. The retention time for betulinic acid and ursolic acid in sample solutions were 8.48 and 9.73mins respectively. The developed method provided good separation with resolution of 4.130. The developed HPLC method was validated in accordance with ICH guidelines. The developed HPLC method was found to be rapid, simple, and precise and provided good resolution of betulinic acid and ursolic acid from other phytoconstituents present in the dried stem bark powder of *Mimusops elengi* Linn. The percentage of betulinic acid and ursolic acid were found to be 97.71 ang 98.65% respectively [21]. Srinivas and Isadore have developed a HPLC method for the analysis of mesembrine type of alkaloids in sceletium plant material. Extraction is done using methanol as solvent. A mobile phase of ammonium acetate in water and acetonitrile is used for the separation of alkaloid constituents. The isolated compounds were characterized by UV, NMR and LC-MS. The method was validated and used to assay the mesembrine-type alkaloids namely 7mesembrenone, mesembranol, mesembrenone, mesembrine and epimesembranol. The calibration curves were found to be linear over the entire concentration range of 400-60,000 ng/ml with correlation coefficients >0.99. The accuracies of the relevant alkaloids were found to be between 94.8 and 103.6% with an inter-day relative standard deviation (RSD) of less than 2.8%. The precision studies showed inter-day RSD's of less than 3%. The recoveries were all within the range of 95 and 105% (RSD<4.5%) and the limits of quantitation (LOQ) and detection (LOD) were found to be 100 and 200 ng/ml respectively [22]. A HPLC method is developed by Rajasekhar and Elango for the estimation of alkaloid content of *Withania somnifera*. Methanol soluble root extract of the plant was subjected to column chromatography to isolate and purify the phyto-constituents. Compounds were characterized as withanone and withaferin-A. Novel HPLC methods were developed for assessment of purity, for standardization and for estimation of the compounds. A GC-MS experiment is performed to confirm the peaks of interest. The method was validated in accordance to USP. The LOD and LOQ values for two standard compounds were confirmed between 400.0 and 1.6µg/ml. The RSD values of less than 2.0% confirmed the precision of the method [23]. Neeraja et al., has developed a HPLC method for the quantitative determination of hyperforin from *Hypericum perforatum*. The hyperforin was separated by using gradient mode consisting of 0.2 % formic acid in water and 0.2% formic acid in methanol at a flow rate of 1.0mL/min. Under these conditions, a plot of integrated peak area versus concentration of hyperforin was found to be linear over the range of 1.0 – 100.0 µg/mL, with a relative standard deviation of 0.16 – 0.88%. The LOD and LOQ values were found to be 10ng and 20ng on column. The determination of the hyperforin content in a commercially available St. John's Wort extracts exhibited a mean content of 2.0 – 27.0 % w/ w. Recovery experiments led to a mean recovery rate of 98.12 ± 0.94 % [24]. A simple, fast and validated HPLC method is developed by Priscila et al., for the quantification of β-Escin in *Aesculus hippocastanum* L. hydroalcoholic extract. HPLC analysis is done using a mobile phase consisting solely of methanol at 1.0mL/min. The method was validated for specificity, linearity, limits of detection and quantification, precision, accuracy and robustness and was proven adequate for quality control of this natural product. The study of the linearity of the method was subjected to the least squares method and obtained a correlation coefficient greater than 0.99 (R2=0.9913). The average recovery values were found to lie within acceptance criteria (98-102%) [25]. Renata et al., has developed a validated HPLC method for the quantification of glycoalkaloids (solasonine and solamargine) in *Solanum lycocarpum* and its extracts. Extraction is done by percolation process using ethanol and water (hydroalcoholic extract) as solvent. HPLC analysis is done using mobile phase consisting of acetonitrile and sodium phosphate buffer (pH 7.2; 0.01M) in a ratio of (36.5:63.5 v/v) at a flow rate of 1.0mL/min. The analytical method was validated and gave good detection response with linearity over a dynamic range of 0.77–1000.00 μgmL−1 and recovery in the range of 80.92–91.71%, allowing a reliable quantitation of the target compounds. Unripe fruits displayed higher concentrations of glycoalkaloids (1.04%±0.01 of Solasonine and 0.69%±0.00 of Solamargine) than the ripe fruits (0.83%±0.02 of SN and 0.60%±0.01 of SM). Quantitation of glycoalkaloids in the alkaloidic extract gave 45.09%±1.14 of Solasonine and 44.37%±0.60 of Solamargine, respectively [26]. A HPLC-DAD method is developed by Gulem et al., for the quantitative determination of galanthamine and lycorine in *Galanthus elwessi* (aerial parts from different localities are used for the analysis). Extraction is done by maceration process. The chromatographic separation was performed using an isocratic system with a mobile phase of trifuoroacetic acid-water-acetonitrile (0.01:90:10) applied at a flow rate 1 mL/min using diode array detector. The content of galanthamine in the aerial parts and bulbs of *G. elwesii* collected from Cimi village (Antalya) was determined as 0.346 and 0.042 %, respectively. The aerial part of G. elwesii collected from Ibradi (Antalya) was found to contain 0.287 % galanthamine, whereas the bulbs contained 0.095 % of this alkaloid. Galanthamine was not detected in the samples of *G. elwesii* growing in Kayrak village (Mersin). Among the tested specimens, lycorine was only found in the bulbs of *G. elwesii* collected from Ibradi (Antalya) and Kayrak village (Mersin) as 0.005 and 0.015 %, respectively [27]. An efficient and reproducible HPLC method is developed by Geison et al., for the simultaneous quantification of main phenolic compounds (chlorogenic acid, isoorientin, orientin and isovitexin) from leaves of *Cecropia* species, *C. glaziovii* and *C. pachystachya*. Extraction is done by infusion process using aqueous solvents. Isolation of the *C*-glycosylflavones was done by column chromatography and the compounds isolated are isoorientin and isovitexin and identified on both species chlorogenic acid (3-*O*-caffeoylquinic acid) and the *O*-glycosylflavonol isoquercitrin. The *C*-glycosylflavone orientin was isolated only from *C. pachystachya*. Chlorogenic acid was the major compound in both species (11.1 mg g-1 of extract of *C. glaziovii* and 27.2 mg g-1 of extract of *C. pachystachya*) and for the flavonoids quantified, isovitexin was the main *C*-glycosylflavonoid for *C. glaziovii* (4.6 mg g-1 of extract) and isoorientin the main one for *C. pachystachya* (17.3 mg g-1 of extract) [28]. Vipul et al., has developed and validated a simple, economic and rapid RP-HPLC method for estimation of piperine in *Piper nigrum* L. HPLC analysis is done using a mobile phase consisting of acetonitrile, water and acetic acid

(60:39.5:0.5). The flow rate was set to 1.0 ml/min with UV detection at 340 nm with run time 10 min and injection volume set at 20µl. The percentage of RSD for precision and accuracy of the method was found to be less than 2%.The percentage recovery of piperine was found to be 99.29% and the developed method was validated in terms accuracy, precision, robustness and recovery. This selective method is found to be accurate, repeatability and effectively used for the Piper extract in marketed sample with better chromatographic conditions. The piperine content in *Piper nigrum* extract was found to be 95.61% [29]. Dada et al., has developed a RP-LC-DAD method for the quantitative determination of protoberberine alkaloids (Jatrorrhizine, Palmatine and berberine) in *Tinospora cordifolia*. Extraction is done using dilute acetic acid in the ratio of 1:40 followed by centrifugation. After isolation the isolated compounds were identified using LC-MS-ESI technique and the quantitative determination of protoberberine alkaloids is done using RP-LC-DAD method. The developed method was validated based on ICH-Q2B guidelines and was found to be accurate, precise and linear over a relatively wide range of concentrations (0.65–83.33 mg mL-1) [30]. Satyanshu et al., has developed a rapid validated reverse phase high performance liquid chromatographic method for the simultaneous determination of three biologically active compounds (Andrographolide, Neoandrographolide and Andrograpanin) from *Andrographis paniculata*. The efficiency of supercritical fluid extraction (SFE) using carbon dioxide was compared with the solid–liquid extraction techniques such as solvent extraction, ultrasound assisted solvent extraction and microwave-assisted solvent extraction with methanol, water and methanol–water as solvents. Chromatographic separation is done using a gradient mixture of acetonitrile (15% solvent A) methanol-water, 60:40 (85% solvent B) as mobile phase at a flow rate of 0.6mL/min. Under the best SFE conditions tested for diterpenoids, which involved extraction at 608C and 100 bars, the extractive efficiencies were 132 and 22 mg/g for Andrographolide and neoandrographolide, respectively [31]. A RP-HPLC method for the estimation of rubiadin from Rubia cordifolia is developed by Khodke et al., Rubiadin was isolated from roots *Rubia cordifolia Linn* and purified. RP- HPLC method for Rubiadin was developed using mobile phase Methanol: water in the ratio of 80:20(v/v). Stationary phase use for this method was Hi-Qsil C18 (250×4.6mm). Using this mobile phase marker gets eluted at Rt 8.657 min. This RP-HPLC method was validated as per ICH guidelines. A linear relationship between peak areas and concentrations was obtained in the range of 10-50µg/mL. Repeatability studies show %RSD to be less than 2%. Excellent recoveries were obtained at each level of added concentration as mean recovery was found to be within 98 to 102% for rubiadin. The LOD and LOQ values were found to be 55.75ng/mL and 184ng/mL. Rubiadin peak gets well resolved from peaks of other chemical constituents [32]. Umashankar et al., has developed a high pressure liquid chromatographic method for the determination of bergenin and afzelechin from different parts of *Bergenia ligulata* Yeo. Extraction is done by percolation process using chloroform and methanol as solvents. Isolation of bergenin and afzelechin from the extracts is done by column chromatography. HPLC method has been developed using a waters C-18 column (250×4.6mm) with photo diode array detector in gradient mode with mobile phase (water: acetonitrile 95:5 to 70:30 in 20min) at a flow rate of 1.0mL/min. The method is sensitive, precise and gives more than 99% recovery of the active constituents. The rhizomes of the plant were found to contain higher concentrations of bergenin (0.907% w/w) and (+) -afzelechin (0.168% w/w) than other parts of the plant [33]. A precise and reversed phase HPLC method is developed by Pothitirat and Gritsanapan for the quantitative analysis of α-mangostin in *Garcinia mangostana* Linn. Extraction is done using soxhlet apparatus with 95% ethanol as solvent. Chromatographic separation was performed using gradient mobile phase consisting of 70-80 % acetonitrile in 0.1 %v/v ortho phosphoric acid at the flow rate of 1mL min-1 with UV detection at 320 nm. The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). The linearity of the proposed method was found in the range of 10–200 µg mL-1 with regression coefficient 0.9999. Intraday and interday precision studies showed the relative standard deviation less than 2 %. Accuracy of the method was determined by a recovery study conducted at 3 different levels, and the average recovery was 100.01 %. The LOD and LOQ were 0.06 and 0.17 µg mL-1, respectively. Two samples of mangosteen fruit rind were separately extracted and analyzed using validated HPLC method. The contents of α-mangostin in the crude extracts and dried powder were within the ranges of 8.36 – 10.04 and 1.84 – 2.47 %w/w, respectively [34]. Kirti et al., has developed a HPLC method for the determination of picrosides content (picroside-I and picroside-II) in the rhizomes of *Picrorhiza kurroa* Royle ex. Benth. Extraction is done by percolation process using 80% methanol as solvent. Quantification of picrosides was performed on waters HPLC system using waters spherisorb reverse phase C-18 column (4.6×250mm, 5µm) and mobile phase; solvent A-(0.05% triflouroacetic acid in water) and solvent B-(1:1 methanol/acetonitrile mixture) solvent A and B are used in the ratio of 70:30 % v/v. The picrosides content was found to be 10.9% [35]. Shinde and Laddha have developed a new isolation technique and validated HPLC method for Khellin a major constituent of *Ammi visnaga* Lam fruits. Extraction is done by solvent extraction followed by partitioning of solvents. The isolated compound was identified by UV visible spectroscopy, IR, MASS, 1H-NMR, 13 C-NMR and TLC. Reverse phase HPLC method has been developed for the quantification of khellin by using an isocratic mobile phase, acetonitrile and water with 0.1%

orthophosphoric acid (40:60v/v)at a flow rate of 1.0mL/min. The wavelength of maximum absorption selected for quantification of khellin was 338nm. The amount of khellin present in the fruits was found to be 1.06% w/w. The developed method has been validated for accuracy, precision and specificity. The calibration plot for khellin was linear in range of 10-60µg/mL. The LOD and LOQ values were found to be 3.03 and 9.09µg/mL respectively. Intraday and interday RSD of retention time and peak area were less than 2%. Recovery was in the range of 98.5-105% with %RSD < 2% [36]. Meena et al., has developed a reliable, accurate and reproducible HPLC method for simultaneous estimation of four furanocoumarins (psoralen, isopsoralen, xanthotoxin and bergapten) in *Psoralea (P.) corylifoia* and *Ammi (A.) majus* plants. Extraction is done using methanol and chloroform as solvents with the help of a sonicator. The furocoumarins were separated simultaneously on a reverse phase Symmetry C8 (150mm×4.6mm) column in isocratic method of methanol, acetonitrile and water solution as mobile phase having flow rate at 0.8 mL/min and detected with UV detector. Maximum psoralen and isopsoralen (Angelicin) were recorded in *P. corylifolia*, whereas maximum 8-methoxypsoralen (xanthotoxin) and 5-methoxypsoralen (bergapten) were found in *A. Majus* [37]. Laxman et al., has developed a high pressure liquid chromatographic method coupled with photodiode array detection for simultaneous determination of eight different marker compounds (ascorbic acid (1), gallic acid (2), methyl gallate (3), chlorogenic acid (4), ethyl gallate (5), ellagic acid (6), rutin (7) and chebulagic acid (8)) in *Phyllanthus emblica*. Extraction (of dried fruit powder) is done using methanol and water (70:30) as solvent with the help of an ultrasonicator. The chromatographic separation was performed on Thermo Scientific BDS HYPERSIL Phenyl reversed-phase column (250mm×4.6mm, 5µm). The mobile phase was consisted of 0.1% ortho-phosphoric acid: Methanol (95:05v/v) (A) and acetonitrile (B) at a flow rate of 1.5 ml/min gradient mode. Regression equations showed good linear relationships (R2 > 0.998) between the peak area of each marker and concentration. The assay was reproducible with overall intra- and inter-day variation of less than 3.4%. The recoveries, measured at three concentration levels, varied from 97.8% to 101.1%. The method was applied to determine the amounts of the marker compounds in dried fruits of *Phyllanthus emblica*, and significant variations in phytoconstituents were observed [38]. A simple, sensitive and precise reversed- phase high performance liquid chromatography (HPLC) method is developed by Farhin et al., for quantitative determination of eugenol from the methanolic extracts of spices viz, dried bud powder of clove, bark powder of *Cinnamomum zeylanicum*, leaves powder of *Cinnamomum tamala*, seed powder of *Myristica fragrans* and leaves powder of *Ocimum sanctum*. The reverse – phase HPLC analysis was carried out using C-18 a mobile phase comprising of methanol, acetonitrile and water in the volume ratio of (10: 50: 40) at a flow rate of 0.7 mL/min in an isocratic system. This method was developed for both standard eugenol and eugenol present in methanolic extracts of various spices. The detection and quantitation of both compounds was carried out at 280 nm. The amount of eugenol in methanolic extracts of five species is found to be 21.91, 6.53, 5.37, 5.93 and 5.64µg/mL respectively. The values of %RSD for intra and inter day precision were less than 2% for the standard. The % recovery for eugenol was found to be 99.8%, which is maximum in *Syzigium aromaticum*[39].

HPTLC methods

Venkatachalapathi and Subban developed a high performance thin layer chromatographic method for the isolation and quantification of lupeol in *Strobilanthes ciliates* Nees. Lupeol was isolated from the petroleum ether extract of aerial parts of *Strobilanthes ciliatus Nees* by column chromatography and identified by IR, NMR, and MS spectral data. It was quantified in the petroleum ether extract by HPTLC method, and found to be 0.16±0.02% w/w [40]. Khushboo et al., has developed a new, simple, sensitive, accurate and precise validated high-performance thin-layer chromatographic method for quantification of psoralen from *Psoralea corylifolia*. The method employed TLC aluminium plates precoated with silica gel 60F- 254 as the stationary phase. Linear ascending development with toluene–ethyl acetate 7.5:2.5 (v/v) as the mobile phase was performed at room temperature (25 ± 2°C) in a twin-trough glass chamber saturated with mobile phase vapour. Compact bands (Rf 0.47 ± 0.02) were obtained for psoralen. Scanning was performed in absorbance mode at 299 nm. Linear regression analysis of the calibration plots showed good linear relationship between peak area and peak height (r2 = 0.99828 and 0.99649) in the concentration range 10–100ng/ spot. The limits of detection and quantification were 8.65 and 26.2 ng/ spot, respectively. The average recovery of the method was 99.73 %. The amount of psoralen in seed powder extract was found to be 0.928% [41]. A high performance thin layer chromatographic method is developed by Yogini et al., for the quantitative determination of catechin from extracts of cashew leaves. Extraction is done by using soxhlet extractor using ethanol and water as solvents. The chromatographic parameters such as solvent system, development time, saturation time, detection wavelength were optimized. The mobile phase toluene: ethyl acetate: methanol: formic acid (6:6:1:0.1v/v/v/v) gave the best resolution for various components. The separation of various components and quantitation of amount of catechin was successfully carried out from extracts of cashew leaves. The aqueous extract of leaves contained a higher amount of catechin as compared to ethanol extract. The presence of tannins and phenolics was visualised as bluish-black bands with

5% alcoholic $FeCl_3$ as visualising agent [42]. A sensitive, selective and precise thin-layer chromatographic method is developed by sweety et al., for the analysis of Karanjin in *Pongamia pinnata*. Extraction is done using ethanol as solvent with the help of sonicator. Separation and quantification was achieved by TLC using mobile phase of ethanolethyl acetate (7:3, v/v), (RF 0.69) on precoated silica gel 60F254 aluminium plates and densitometric determination was carried out in reflection/absorption mode at 260 nm. The calibration curve was linear in the concentration range of 0.1µg-1.2µg spot-1. The method was validated for precision, repeatability and accuracy. The LOD and LOQ values were found to be 0.0101µ/mL and 0.0739µg/mL respectively. % Recovery values were found to be 97.19 ± 1.204 which shows high extraction efficiency of karanjin. The karanjin content in Pongamia pinnata was found to be 2.37± 0.66 in µg/g sample [43]. Abid et al., has developed a simple, sensitive, and specific thin layer chromatography (TLC) densitometry method for the simultaneous quantification of strychnine and brucine in the seeds of *Strychnos nuxvomica*. The method involved simultaneous estimation of strychnine and brucine after resolving it by high performance TLC (HPTLC) on silica gel plate with chloroform–methanol–formic acid (8.5:1.5:0.4 v/v/v) as the mobile phase. The method was validated as per the ICH guidelines for precision (interday, intraday, intersystem), robustness, accuracy, limit of detection, and limit of quantitation. The relationship between the concentration of standard solutions and the peak response was linear within the concentration range of 50–1000 ng/spot for strychnine and 100–1000 ng/spot for brucine. The method precision was found to be 0.58–2.47 (% relative standard deviation [RSD]) and 0.36–2.22 (% RSD) for strychnine and brucine, respectively. Accuracy of the method was checked by recovery studies conducted at three different concentration levels and the average percentage recovery was found to be 100.75% for strychnine and 100.52% for brucine, respectively [44]. Arvind et al., has developed a high performance thin layer chromatographic method for quantitative determination of yohimbine alkaloid in different parts of *Rauvolfia tetraphylla*. Methanol is used as solvent for extraction and the methanol extracts of leaves; stem and roots of *Rauvolfia tetraphylla* and yohimbine as standard are used for the study. The compression was done by HPTLC. The linear calibration ranges were 10-1000g/ml for yohimbine. Yohimbine was detected only in the leaves of plant in 6.11%. The HPTLC methods were successfully validated and applied to the quantization of yohimbine [45]. A validated HPTLC densitometric method is developed by Homa et al., for the estimation of aucubin in Vitex agnus-castus L. Extraction is done using methanol as solvent. Chromatographic separation was performed using silica gel high performance thin-layer chromatography (HPTLC) plates with ethyl acetate-methanol-water 77: 15: 8 as mobile phase. Chromatograms were visualized using *p*-dimethylaminobenzaldehyde as reagent. Aucubin Rf-value was about 0.5 and spots were scanned at 580 nm through a mercury lamp. By using this method, the amount of aucubin was found 43.5mg/100 g of dried plant fruits. The method was validated for selectivity, linearity (r2 = 0.997, 20-100 μg/mL), precision (intra-day < 4.9, inter-day < 7.2) and accuracy measured via determination of recovery (95-98%). The limit of detection and limit of quantization were found 6.6 and 20μg/mL, respectively. This methodology was found to be precise with respect to the validation parameters [46]. Joshi et al., has developed a high performance thin layer chromatographic method for the simultaneous estimation of quercetin, kaempferol and asiatic acid in leaves of two chemotypes of *Centella asiatica*. Successive extraction is done using methanol, hydromethanol and water by cold maceration method. The HPTLC method was developed using a suitable mobile phase Toluene: Ethyl acetate: Chloroform: Formic acid (6:6:4:1). The Rf values for asiatic acid, quercetin, and kaempferol was 0.33, 0.44 and 0.55 respectively. The linear regression analysis for the calibration showed a linear relationship with r2= 0.9764 and 0.9823 for quercetin and kaempferol respectively in the concentration range of 100-1000ng/band. In case of Asiatic acid, the linear regression analysis data for the calibration plots showed a linear relationship with r2=0.9996 in the concentration range of 100-500ng/band. The average recovery for quercetin was 98.60% for kaempferol was 90.08% and for asiatic acid was 97.66%. System suitability parameter was evaluated by spotting five replicates of all three standards. The %RSD was found to be below 2% with respect to peak area as well as Rf value. Robustness of method was evaluated by changing mobile phase concentration from toluene: ethyl Acetate: chloroform: formic Acid (6:6:4:1) to (5:5:5:1), for which %RSD of peak areas was found to be below 10% [47]. A high performance thin layer chromatography method is developed by pandey et al., for the identification and determination of protocatechuic acid present in greater cardamom fruit extracts. The powdered drug was subjected to extraction by soxhlet apparatus using methanol and acetone separately as well as petroleum ether (40-60), chloroform, methanol and water successively. The extracts were screened for presence of various phytoconstituents using preliminary chemical tests. Protocatechuic acid was estimated in methanol and acetone extract by HPTLC method. Detection and quantification was performed at wavelength 254nm. The acetone and methanol extracts were found to contain 1.04846 and 0.8634 %w/w Protocatechuic acid respectively by using validated method [48]. A simple, robust, reproducible, rapid, sensitive and accurate high performance thin layer chromatographic method is developed by Chinthala et al., for quantitative determination of nitidine from roots and plant tissue culture extracts of *Toddalia asiatica* Linn. Extraction is done using 70% methanol by refluxing for 20minutes. Nitidine was estimated at 332 nm by densitometry using Silica gel 60 F254 as stationary phase and chloroform: methanol (7:1, v/v), and as mobile phase. Linearity was observed in the concentration range of 25 - 200 ng/spot for nitidine. The limit of detection and limit of quantitation were found to be 0.026 and 0.086 ng/spot respectively for nitidine. Developed method was validated according to the ICH guide- lines with respect to precision, accuracy, specificity and robustness [49]. Preeti et al., has developed a simple, precise and rapid high-performance thin layer chromatographic method for the estimation of phyllanthin in *Phyllanthus amarus*. Extraction is done using soxhlet apparatus and methanol as solvent. Separation of phyllanthin was carried out on silica gel 60 F254 layers eluted with hexane: ethyl acetate (2:1), and the analytes were visualized through colour development with 10% concentrated sulphuric acid in ethanol. Scanning and quantification of spots was performed at 200 nm. Best results were obtained using Hexane: ethyl acetate (2:1) as mobile phase. The compound with Rf value of 0.24±0.03 was identified as phyllanthin. Linearity was found over the concentration range of 2-12ng/spot with correlation coefficient of 0.985. The recovery rate was 98.5%. The LOD and LOQ values were found to be 60 and 120ng/spot respectively. The % RSD values were found to be less than 2% [50]. Gohari et al., has developed a high performance thin layer chromatographic method for the isolation and quantificative analysis of oleanolic acid from *Satureja mutica* Fisch. Dried aerial parts of *S. mutica* were successively extracted with diethyl ether. This extract was used for further isolation on silica gel column chromatography to obtain compound. Isolated compound was identified based on the spectral data of 1H-NMR, 13CNMR and Mass spectroscopy. Densitometric analysis of the developed plate of TLC was carried out to quantify the oleanolic acid using TLC scanner. Isolated compound was structural elucidated as oleanolic acid and its NMR data showed good agreement with the reference data mentioned in literature. Spectrodensitometric analysis showed that the band characterized by absorption maximum of oleanolic acid was placed at λmax = 270 nm without derivatization. The contents of oleanolic acid are calculated 17.5 mg in 100 g of *S. mutica* leaves based on dry weight [51]. Saikat et al., has developed a high performance thin layer chromatographic method for the detection and estimation of alpha-amyrin, beta-sitosterol, lupeol and n-triacontane in two medicinal plants (*Leptadenia reticulata and Pluchea lanceolata*). Extraction is done using methanol and chloroform (1:1v/v) as solvent with the help of ultrasonicator. Chromatographic separation was performed on TLC aluminium plates precoated with silica gel $60F_{254}$ using a suitable mobile phase. The developed HPTLC method has been validated and used for simultaneous quantitation of the four components from the methanolic extracts of whole plant powders of *Leptadenia reticulate* and

Pluchea lanceolata. The linearity ranges were found to be 0.1-1.0µg/band for alpha amyrin, beta sitosterol and lupeol. The linearity range for n-triacontane is 0.1-10.0µg/band. The LOD and LOQ values were found to be 0.03µg/band for alpha amyrin, betasitosterol, lupeol and 0.10µg/band for triacontane. The LOD and LOQ values were found to be 0.30 and 1.0µg/band respectively. The %RSD values were found to be less than 2 which indicate that all peaks are well resolved [52]. Priti and Kilambi developed a validated high performance thin layer chromatographic method for the estimation of gymnemic acids through gymnemagenin in *Gymnema sylvestre*. Extraction is done by refluxing for 2 hours in 2.5N 50% methanolic hydrochloric acid. Separation and quantitation is done through HPTLC method. As gymnemagenin, a common aglycone of gymnemic acids, lacks in conjugation, very poor UV absorption is observed. So post-derivatization method was used for quantification of gymnemagenin. Linearity was observed in the range of 180 – 1440 ng/spot. Thus the method was found to be more sensitive where gymnemagenin was quantified at nanogram level. The method was validated as per ICH guidelines and successfully applied for quantification of gymnemagenin from plant leaf powder, extract and poly herbal marketed formulation. Percentage recovery was found to be 98.4 ± 1.0% [53]. Aldon et al., has developed a precise, accurate and reproducible high performance thin layer chromatographic method for simultaneous quantification of boeravinone B, eupaliptin-3-O-β-D-galactopyranoside and β-sitosterol from *Boerhavia diffusa* Linn. Extraction is done using soxhlet apparatus followed by chromatographic separation on HPTLC silica gel 60 F254 pre-coated plates using Toluene: Ethyl Acetate: Methanol as mobile phase. Quantification was carried out in binary mode at 342 nm (Boeravinone B and Eupalitin-3-O-β-D-galactopyranoside) and 580 nm (β-sitosterol). Linear responses for boeravinone B, Eupalitin-3-O-β-D-galactopyranoside and β-sitosterol were obtained over the concentration ranges of 15-105 µg/mL, 12-84 µg/mL and 12-84 µg/mL[54]. A high performance thin layer chromatographic method is developed by Rathee et al., for simultaneous quantification of bergenin, catechin, gallicin and gallic acid and β-sitosterol in *Bergenia ciliata*. Quantification by HPTLC is done using the solvent System of Toluene: Ethyl acetate: Formic acid (6: 6: 1, v/v/v). β-Sitosterol was quantified from petroleum ether extract using the Solvent System of Toluene: Methanol (9: 1, v/v). The methods were validated using ICH guidelines in terms of precision, repeatability and accuracy. The densitometric methods were found to be precise with RSD for intra-day in the range of 0.15–0.52 and for interday in the range of 0.26–0.61 for different concentrations of Bergenin, (+)-Catechin, Gallicin, Gallic acid and β-Sitosterol respectively. The linearity ranges for Bergenin, (+)-Catechin, Gallicin and Gallic acid were found to be the same (160 –720 ng/spot) with correlation coefficients (*r*-values) of 0.999, 0.997, 0.998 and 0.996

respectively. The linearity range for β-Sitosterol was 80–480 ng/spot with correlation coefficient (*r*-values) of 0.995. Instrumental precision was 3.39, 3.36, 3.05, 1.20 and 0.85 (% RSD) for Bergenin, (+)-Catechin, Gallicin, Gallic acid and β-Sitosterol respectively. Accuracy of the method was checked by conducting recovery studies at three different levels for both the compounds and the average percentage recoveries obtained were 100.38, 101.45, 102.38, 99.9 and 99.92% respectively for Bergenin, (+)-Catechin, Gallicin, Gallic acid and β-Sitosterol. The amount of Bergenin, (+)-Catechin and Gallic acid was 0.22, 0.063 and 0.25 % w/w respectively whereas Gallicin was not in detectable amount [55]. Savitha and Arivukkarasu developed a high performance thin layer chromatography densitometric method for the determination of phytocompounds from *Terminalia chebula*. Extraction is done using soxhlet apparatus and methanol as solvent. Desired resolution is achieved using toluene: ethyl acetate: formic acid: methanol (4.3:4.3:1:1.2:0.3, V/V/V/V) was used as a mobile phase. Detection and quantification were performed densitometrically at Lambda 254nm. The Rf values of standards were 0.78 for TA, 0.74 for GA and 0.63 for EA. The % recovery values were found to be 16.13, 279.42 and 22 µg/mL for tannic acid, gallic acid and ellagic acid respectively [56]. Vijayalaxmi and Ravindhran developed a high performance thin layer chromatographic method for the quantitative determination of gallic acid in ethanolic root extract of *Diospyrus ferrea* and *Aerva lanata*. Roots of plants are macerated and extracted with hexane, chloroform, methanol, ethanol and aqueous for 48hours in a soxhlet assembly. The Toluene-Ethyl acetate-Formic acid-Methanol (3: 3: 0.8: 0.2) was employed as mobile phase for gallic acid. The ethanolic root extract of *A. lanata* and *D. ferrea* illustrated the presence of gallic acid with different range of Rf from 0.38 to 0.74. The gallic acid with the Rf value 0.48, 0.47 was present in *Aerva lanata* and 0.46, 0.47 was reported in *Diospyrus ferrea* corresponding as that of standard. The gallic acid with the Rf value 0.47 showed their joint presence in both the root extract. Maximum amount of gallic acid has been observed in root of *Diospyrus ferrea* compare to that of *A. lanata*. Detection and quantification were performed by densitometry at $\lambda = 277$ nm. The average recovery of gallic acid was found to be 47.50µg/ml in *Diospyrus ferrea* and 2.61µg/ml in *Aerva lanata* respectively. Validated method showed linear response over concentration range of 100 to 700ng. The LOD and LOQ values were found to be 100, 300 ng/spot respectively [57]. Neena et al., has developed a high performance thin layer chromatographic method for the estimation of p-coumaric acid and triacontanol in *Stereospermum suaveolens* Roxb. Extraction (of powdered roots) is done using methanol as solvent by cold maceration technique. The solvent system is composed of toluene–ethylacetate–formic acid for triacontanol (9:1:0.2, v/v/v); and toluene–ether–acetic acid for *p*-coumaric acid (5:5:0.1, v/v/v) for

HPTLC studies. Studies on root as well as stem samples showed characteristic bands of triacontanol and *p*-coumaric acid at Rf 0.51 and 0.42, respectively. The amount of triacontanol varied from 0.035–8.472mg/g in the root samples while the amount of p-coumaric acid was in the range 0.081–0.203 mg/g. The limit of detection for triacontanol was 100 ng/spot whereas that for *p*-coumaric acid was 10 ng/spot [58]. Alok et al., has developed a high performance thin layer chromatographic method for the quantitative estimation of berberine content in *Berberis asiatica*. Ethanolic extracts of various plant samples were analyzed with standard berberine (purified) in different concentrations 1-5µl. The peak areas of each standard and test samples were obtained from the system and a calibration graph was plotted between concentrations vs. Peak area. The plant samples belonging to lowest altitude region was found to possess maximum concentration of berberine which was 2.94% however berberine content was non-linearly distributed among the other samples of higher altitudes [59]. Daya and Patel developed a high performance thin layer chromatographic method for the quantitative determination of gallic acid in fruits and leaves of *Careya arborea*. Extraction is done using methanol and ethyl acetate as solvents. The thin layer chromatography plate was spotted with gallic acid and fruits and leaves extract of *C. arborea*. The extract of fruit and leaves shows the Gallic acid spot at Rf: value 0.68 using mobile phase ethyl acetate: toluene: formic acid (8:2:0.3, v/v). Hence, the gallic acid was selected as a marker. The gallic acid was quantified using HPTLC technique in *C. Arborea* fruit ethyl acetate extract (CFE), *C. arborea* fruit alcoholic extract (CFA), *C. arborea* leave ethyl acetate extract (CLE) and *C. arborea* alcoholic extract of leaves (CLA) of *C. arborea*. The percentage (w/w) amount of gallic acid was found to be 2.11%, 1.2%, 0.54% and 0.48% in CFE, CFA, CLE, and CLA of *C. arborea*, respectively [60]. A simple and rapid high performance thin layer chromatographic method is developed by Hamrapurkar et al., for the estimation of piperine in *Piper nigrum* and *Piper longum*. Extraction is done using soxhlet and supercritical fluid extraction technique. Piperine was isolated from the extract using column chromatography. Characterization of compound was done by spectroscopic technique. The % w/w yield of piperine in Supercritical fluid extract was 8.76 for *P.nigrum* and for *P.longum* 4.96. Where as, in case of soxhlet extraction % w/w yield for *P.nigrum* 8.13 and for *P.longum* % w/w was 4.32. Time required for extraction of piperine by soxhlet method was 8.0hr whereas for supercritical fluid extraction only 0.5hr. This shows supercritical fluid extraction has more extraction efficiency and reduced extraction time than soxhlet extraction method. Also the results showed % w/w yield of piperine was found to be higher in *P.nigrum* than *P.longum* [61]. A simple, accurate and precise high performance thin layer chromatographic method is developed by Prajapathi et al., for the simultaneous estimation of quercetin and rutin in *Saraca* *asoca*. Fresh bark of *Saraca asoca* is extracted with methanol using cold maceration technique. HPTLC analysis was performed using toulene: ethyl acetate: methanol: formic acid (8:8:3:1) as mobile phase. The linear regression analysis data for the calibration plots showed a good linear relationship in the concentration range of 100-1000ng/band with correlation coefficient of 0.993 and 0.99 for quercetin and rutin respectively. The average recovery for quercetin was 98.593% and 97.66% for rutin [62]. Divya et al., has developed a high performance thin layer chromatographic method for the estimation of lignans (sesamin and sesamolin) as markers in *Sesamum indicum*. Extraction is done using methanol as solvent. The HPTLC analysis is done using benzene–chloroform (50:1, v/v) as mobile phase. For the confirmation of identity and purity of bands, the bands corresponding to sesamin and sesamolin were scrapped and eluted with chloroform, and 1H NMR (Avance DPX 300, Bruker, UK) and MS (MS Route JMS 600H, JEOL, Japan) spectra were acquired. The amounts of sesamin and sesamolin were quantified using calibration curves plotted with the reference compounds. The Rf values obtained for sesamin and sesamolin were 0.19 and 0.29 resspectively. The LOD and LOQ values were found to be 0.08 and 0.24µg for sesamin, 0.02 and 0.06µg for sesamolin. The % RSD of inter and intraday precision was found to be between 95.7-102.0%. The contents of sesamin and sesamolin are found to be 0.72 and 0.40% respectively [63]. Suhagia et al., has developed a simple, sensitive and precise high performance thin layer chromatographic method for the quantitative analysis of oleanolic acid in seeds of *Acyranthes aspera* Linn. Extraction is done using 95% ethanol by refluxing process. Chromatographic separation is done using hexane-ethylacetate-acetic acid (8+4+0.2v/v/v) as mobile phase. The method was validated for precision, repeatability and accuracy. A polynomial relation was obtained for oleanolic acid in the range of 200-1200ng/spot. The method was found to be precise, with relative standard deviation (RSD) values for intraday analyses in the range of 1.02-3.71%. The method was further applied to study the effect of germination of seeds of *A.aspera* on the content of oleanolic acid. The content of oleanolic acid in the non-germinated and germinated seeds of *A.aspera* was found to be 0.343 ± 0.0071w/w and 0.625 ± 0.018%w/w respectively [64]. Kulkarni and Khatwani developed a simple, precise, accurate and reproducible high performance thin layer chromatographic method for the estimation of wedelolactone in *Eclipta alba*. Isolation of wedelolactone from the extract is done using column chromatography. Characterization of isolated wedelolactone is done by UV spectrometry and IR studies. HPTLC analysis is done using toluene: ethyl acetate (9:1) as mobile phase. This system was found to give compact spots for wedelolactone (RF value of 0.30 ± 0. 3) with a linearity range of 1 to 80µg per spot. The proposed method was applied to evaluate efficiency of different methods of extraction i.e. percolation, maceration, hot solvent extraction (Soxhlet apparatus), supercritical fluid extraction, microwave, orbital shaker bath and sonication method for extraction of wedelolactone from *Eclipta alba*. The highest percentage of wedelolactone was found in extract prepared by Soxhlet extraction (0.48% w/w) and lowest in case of super critical fluid extraction [65]. Uma et al., has developed a high performance thin layer chromatographic method for the estimation of flavonoids (ferulicacid, Gallicacid, isoquercitin, quercitin) in *Boerhavia diffusa* Linn. Extraction is done using soxhlet extractor and methanol as solvent. HPTLC was performed with methanol, toluene: ethyl acetate, formicacid, 5:4:0.2(V/V/V) as mobile phase, in a camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 minutes. The Rf values were found to be 0.02, 0.32, 0.76 and 0.90 for gallic acid, ferulic acid, Isoquercetin and quercetin. Rutin and caffeic acid are not detected. The concentration of different flavonoids was found to be 49.9, 0.50, 0.05 and 8.51µg/g for gallic acid, ferulic acid, isoquercetin and quercetin respectively [66]. A high performance thin layer chromatographic method is developed by Sarfaraj et al., for the simultaneous quantification of two terpenoid compound lupeol and steroid compound stigmasterol in *Abutilon indicum*. HPTLC analysis is done using a mobile phase of toluene: methanol: formic acid (7.0:2.7:0.3, v/v/v) and densitometric determination of these compounds was carried out at 530nm in reflectance/absorbance mode. Compact bands for lupeol and stigmasterol were obtained at Rf 0.52±0.02 and 0.28±0.05. The limit of detection (45 and 18ng/band), limit of quantification (135 and 54ng/band), recovery (98.2-99.7% and 97.2-99.6%) and precision (< 2.18 and 1.91). Linearity range for lupeol and stigmasterol were 100-1000(r2=0.9994) and 50-500ng/band (r2=0.9941) and the contents were estimated as (0.59±0.10%) and (0.83±0.10) % w/w respectively [67].

HPLC and HPTLC methods

Jayaprakasan et al., has developed two simple and precise HPLC and HPTLC methods for the estimation of stigmasterol from *Acalypha indica* root extracts. Extraction is done using different solvents like petroleum ether, chloroform and methanol by continous hot percolation. HPTLC analysis is done using mobile phase system comprising of toluene: methanol, 9:1 % v/v. After development, the plates were scanned and quantified at 525nm. Linearity was found in the concentration range of 100-900 ng/spot (r=0.9920). Limit of detection was found to be 70ng/spot and limit of quantification was found to be 100 ng/spot. For HPLC method, the optimized mobile phase system used was methanol: water (99:1%v/v) with the flow rate of 1ml/min and the detection wavelength was 202 nm. The calibration curve was obtained in the range 20-60 µg/mL. The slope, intercept and correlation coefficient values were found to be 3264.29, - 4675.4 and 0.9927 respectively. Limit of detection and limit of quantification were found to be 5µg/ml and

20µg/ml respectively [68]. Wasim et al., has developed both HPLC and HPTLC methods for the quantitative characterization of emodin and chrysophanol from *Rheum emodi*. The extraction is done using methanol as solvent by with the help of a sonicator. The simultaneous methods were found linear r2 =0.991 in a wide range (10–800ng spot with HPTLC, 5–500 mg/mL with HPLC) precise, accurate and robust for both the drugs. The robustness of methods was proved by applying the Box–Behnken response surface design software and other validation parameters evaluated were satisfactorily met. The analysis of emodin and chrysophanic acid in *R. emodi* samples using HPTLC showed 3350 and 7060 mg/ kg whereas HPLC 3380 and 7800 mg /kg, respectively [69].

LC-MS methods

A HPLC-UV and ESI-MS combined method is developed by Olivia et al., for simultaneous characterization and quantification of phenolic compounds in *Thymus x citriodorus*. The ethanolic extract of *T. x citriodorus* was analyzed by reversed phase HPLC. The method of analysis was also validated concerning its linearity, instrumental and method precision (for repeatability, immediate precision and intermediate precision) and accuracy (absolute recovery study) and satisfactory results were obtained. The technique was combined with electrospray mass spectrometry in order to identify the phenolic compounds and the structure of the main phenolics was also confirmed by NMR analysis. The extract was rich in rosmarinic acid (10.4 ± 0.6 mg/g extract) that is a widespread phenolic acid in Thymus plants, but also in luteolin-7-O-α-glucuronide (12 ± 2 mg/g extract), that was herein reported in Thymus for the first time. Other novel compounds comprised one eriodictyol dihexoside with O-glycosidic linkages, two eriodictyol-O-monohexosides, one quercetagetin dimethyl ether-O-hexoside, one naringenin-O-hexoside and chrysoeriol-7-β-O-glucoside [70]. A liquid chromatography-electro spray time of flight mass spectrometry method is developed by Ana et al., for the estimation of capsaicin and dihydrocapsaicin from *capsicum* fruits. Dried tissue samples were extracted using acetonitrile as solvent with the help of an orbital shaker. Chromatographic separation was achieved with a reversed-phase using a gradient of methanol and water. Quantification was done using as an internal standard (4,5-dimethoxybenzyl)-4-methyloctamide, a synthetic capsaicin analogue not found in nature. Analytes were base-peak resolved in less than 16 min, and limits of detection were 20 pmol for capsaicin and 4 pmol for dihydrocapsaicin. The intraday repeatability values were lower than 0.5 and 12% for retention time and peak area, respectively, whereas the interday repeatability values were lower than 0.6 and 14% for retention time and peak area, respectively. Analyte recoveries found were 86 and 93% for capsaicin and dihydrocapsaicin, respectively. The method developed has been applied to the identification and quantification of capsaicin and dihydrocapsaicin in fruit extracts from different

Capsicum genotypes, and concentrations found ranged from 2 to 6639 mgkg-1[71]. Kamel et al., has developed a HPLC-MS method for the isolation, characterization and quantification of stilbenes from carex species. The stilbene contents of five unexplored *Carex* species were investigated- *Carex capillacea*, *Carex hirta*, *Carex buchananii*, *Carex cuprina*, *Carex glauca*. Extraction is done by grinding the plant material with methanol. High performance liquid chromatography coupled to mass spectrometry (HPLC–MS) and NMR spectroscopy (NMR) were used to identify the structures. A novel stilbene oligomer, carexinol A, was isolated together with five previously known stilbenes: resveratrol-diglucoside, miyabenol A and C, kobophenol A and α-viniferin. Quantification was performed by HPLC-DAD analysis on an Agilent® 1100 Series system. The total concentrations of identified stilbenes were determined at 3.5mg/g for *C.capillacea*, 7.6mg/g for *C.buchananii*, 2.8mg/g for *C.hirta*, 8.9mg/g for *C.grauca* and 5.0mg/g for *C.cuprina* [72].

GC-MS methods

A GC-MS method is developed by Revathy et al., for the quantification of phenolic compounds (cyano acetyl urea, 4-(2-methyl amino) ethyl pyridine (betahistidine) in *Acalypha alnifolia* Klein Ex wild. Preliminary phytochemical analysis has been carried out by using standard methods. The powdered plant leaf was packed in small thimbles separately and extracted successively with different solvents such as petroleum ether, chloroform, acetone and methanol in the increasing order of polarity using soxhlet apparatus. The GC – MS analysis was carried out using a Clarus 500 Perkin Elmer (Auto systemXL) Gas Chromatograph equipped and coupled to a mass detector. GCMS have been undertaken for screening compounds. Quantification of phenolic compounds shows acetone leaf extract having much more than other solvent extracts. Nine different compounds were identified from the acetone extracts when taken for GC-MS. Acetone and methanol extracts outlined the tannin content in A.alnifolia leaf as 28.62and 18.39mg gallic acid equivalent /gram respectively [73]. Gheewala et al., has developed a GC-MS method for the phytochemical evaluation of total glycoalkaloid (solasodine, solasonine) content of dried fruit of *Solanum nigrum* Linn. Extraction of powdered sample plant material is done with 200 ml of 5% aqueous acetic acid. Crude extract of dried fruit of *solanum nigrum* was analyzed for the presence of the phytoconstituents with the use of analytical methods like HPLC and GC-MS. HPLC analysis is done using mobile phase consisting of 0.05 M acetonitrile: ammonium di-hydrogen phosphate buffer (30:70 v/v) and solvent flow rate- 1.5 ml/min. Shimadzu GC-MS system operating at an ionization voltage of 70 eV with ion source temperature of 180 °C [10]. Samples were analyzed with the help of fused-silica capillary column (15 m- 0.20 mm). 1µl sample was taken for GC/MS analysis. From this study, Solasonine was the steroidal glycoalkaloid which had higher content

(5.85 mg/g) than other relative constituents and aglycone solasodine was present in 75.94% which was significantly higher than solanidine [74]. Senthil kumar and Veerappa developed and validated GC-MS methods for the determination of leaf and root of *Delonix alata* (L.) Gamble. The quantitative determination of the chemical compounds was based on the comparison of peak areas of samples with those in GCMS library. A total 50 compounds of leaf, 45 compounds of root were identified from the methanolic extracts of *Delonix elata.* The identified compounds of leaf, the highest % Peak area of 7.06 is Quercetin (Retention time 0.00 - 39. 93), rutin % Peak area of 11.78 is rutin (Retention time 0.00 - 39.93), the identified compounds of flavonoids. A total 45 compounds of root, 45 compounds were identified from the methanolic extracts of *Delonix elata.* The identified compounds of root, the highest % Peak area of 32.54 is β – amyrin (Retention time 0.00 - 39. 94), hesperitin % Peak area of 7.06 is Rutin (Retention time 0.00 - 39. 93), the identified compounds of flavonoids [75]. Murat et al., has developed a GC-MS method for the quantitative determination of l-hyoscyamine in *Hyoscyamus reticulatus* L. dried and powdered roots and leaves of *H. reticulatus* L. were extracted with methanol in a Soxhlet apparatus for 2h. The hyoscyamine content of leaves and root samples of *Hyoscyamus reticulatus* L. determined by capillary GC-MS was found to be 0.036 ± 0.004% in the leaves and 0.056 ± 0.011% in the roots as the predominant compound. The limit of detection was 3.125μg/mL and the limit of quantification was 6.25μg/mL for hyoscyamine. This method has been shown to be linear and sensitive [76].

NMR methods

Neha et al., has developed a sensitive and reliable proton magnetic resonance spectrometric method for the isolation and quantification of pinitol from *Argyrolobium roseum* plant. Extraction is done by percolation process using 95% ethanol as solvent. Pinitol in pure form was isolated from the ethanolic extract of A. roseum by repeated column chromatography over silica gel followed by crystallization in methanol. Pinitol isolated from the plant was identified on the basis of 1H-NMR, 13C-NMR, experiments and mass spectral data. Validation of the method was carried out by preparing a known concentration of an artificial mixture of pinitol and pyrazinamide. The recovery of pinitol in the mixture was in the range of 98.5–101.3% [7].

Other Hyphenated analytical methods

Yong et al., has developed ultra high performance liquid chromatography method coupled with electrospray ionization quadrupole time of flight mass spectrometry for the qualitative and quantitative estimations of chemical constituents from *Curculigo orchioides.* Dried *Curculiginis* Rhizoma powder (5.0 g) was preciselyweighed and ultrasonically extracted with 20-fold 70% ethanol in a conical flask. Qualitative analysis was performed on a Waters acquity UHPLC by gradient elution with mobile phase of 0.1% formic acid and acetonitrile. Quadrupole TOF/MS in either full scan mode or extracted ion mode was used for qualitative and quantitative analysis of the constituents. Eight phenolic glycosides including 5-hydroxymethylfurfural (HMF), 2-hydroxy-5-(2-hydroxyethyl) phenyl-β-d-glucopyranoside (HPG), anacardoside (ACD), orcinol glucoside (OGD),orcinol-1-O- β-d-apiofuranosyl-(1 → 6)- β-d-glucopyranoside (OAG), 2,6-dimethoxybenzoic acid (DBA),curculigoside (CUR) and curculigine A (CCL) were quantitated [77]. An UPLC-DAD-MS method is developed by Tao et al., for the estimation of diosgenin in *dioscorea* species like (*D.zingiberis, D.septemloba, D.collettii* and *Heterosmilax yunnanensis*). Powdered samples are extracted with 15mL of methanol by means of sonication at room temperature for 0.5 h. Ultra-performance liquid chromatography (UPLC) coupled with diode array detection (DAD) and electrospray ionization mass spectrometry (ESI-MS) method was developed for identification and determination of diosgenin in various plants. A comprehensive validation of the developed method was conducted. The present method presented good sensitivity, precision and accuracy. Diosgenin was found in three *Dioscorea* species and one Heterosmilax species, namely *D. zingiberensis*, *D. septemloba*, *D. collettii* and *H. Yunnanensis* [78]. Ping et al., has developed an UPLC-ESI-MS method for the identification and determination of aconitum alkaloids in *Aconitum* herbs. Chromatographic separations were achieved on a C-18 column and peaks were detected by mass spectrometry in positive ion mode and selected ion recording (SIR) mode. In quantitative analysis, the six alkaloids showed good regression, (r) > 0.9984, within the test ranges. The lower limit quantifications (LLOQs) for mesconitine, aconitine, hypaconitine, benzoylmesaconitine, benzoylaconitine, and benzoylhypaconitine were 1.41, 1.20, 1.92, 4.28, 1.99 and 2.02 ng·mL−1, respectively. Recoveries ranged from 99.7% to 101.7%. The validated method was applied successfully in the analysis of the six alkaloids from different samples, in which significant variations were revealed [79]. Chromatographic fingerprint of *Ixora coccinea* methanolic flower extract is performed by Sumathy et al., using different analytical method like HPLC, HPTLC, GC-MS. Fresh flowers were shade dried, powered (80% coarse: 20% fine) and subjected to successive (petroleum ether, ethyl acetate and methanol) extraction by hot continuous method using Soxhlet's apparatus. Chromatographic analysis was performed with 0.5%aqueous solution of Methanol (HPLC Grade) as mobile phase at flow rate of 1 ml /min. HPTLC analysis is done using the solvent system Chloroform: Methanol (95:5). GC-MS technique was used in this study to identify the phytocomponents present in IC methanolic flower extract HPLC analysis of *Ixora cocccinea* methanolic flower extract revealed the presence of Biochin-A, Myricetin, Quercetin, Rutin, Diadzein and formononetin, HPTLC fingerprint revealed the presence of ursolic acid and

GC-MS with 24 phytochemicals among which some are of biological importance [80]. Jose et al., has developed a qNMR and LC/MS-MS method for the simultaneous identification and quantification of green tea catechins. This approach utilizes computer-assisted1H iterative Full Spin Analysis (HiFSA) and enables rapid pro-filing of seven catechins in commercial green tea extracts. The qHNMR results were cross-validated against quantitative profiles obtained with an orthogonal LC–MS/MS method. This report describes the development and application of an efficient qNMR method for the simultaneous analysis of seven chemical markers in crude extracts of green tea, produced by non-fermented leaves of *Camellia sinensis* (L.) Kuntze. The green tea phytoconstituents selected for this study (Fig. 1)comprise seven catechins known for their antioxidant major catechins found in green tea products are (−)-epigallocatechin-3-O-gallate (EGCg), (−)-epigallocatechin (EGC),(−)-epicatechin-3-O-gallate (ECg), and (−)-epicatechin (EC). Other polyphenols such as (+)-catechin (C), (−)-gallocatechin (GC), and (−)-gallocatechin-3-O-gallate (GCg) are also present, although in smaller quantities [81].

CONCLUSION

Due to chemo-diversity of secondary metabolites and since any kind of pharmacological activity might be found, the role of analysis in herbal drugs is fundamental. Several extraction, purification or separation processes can be applied but the choice of the best methodologies has to be done. The present review helps us to develop a novel, and relatively simple and reproducible method for isolation, characterization and quantitative determination of major constituents in plants. Based on the results obtained we can know whether the developed method can be applied for the analysis or not.

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