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REVIEW ARTICLE

Significance of TLC and HPTLC in Phytochemical Screening of Herbal Drugs

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Abstract: Plants provide the most of the pharmacologically active principles like alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, tannins, essential oils to humans. A large number of the medicinal plants were used by traditional and folk medicinal practitioners for various ailments. The active principles in them are detected by qualitative chemical tests for each category of phytoconstituents. Many chromatographic methods help in separation of the complex mixtures into individual phytoconstituent. Even though Thin Layer Chromatography (TLC) and HPTLC are rapid and easy methods, the selection of stationary phase and solvent system is always a time-consuming process. The general solvent systems for the specific category of secondary metabolites are available in various literatures, but for specific phytoconstituent one should do trial and error method to identify a suitable solvent system. This review article aims to minimize the valuable time of both beginning and experienced researchers by providing well-organized information about TLC and HPTLC in phytochemical screening.

Keywords: *TLC, HPTLC, Solvent system, Extraction, Phytochemical analysis, Herbal drugs.*

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Introduction

India has a very long, safe and continuous usage efficacy of many plant-based drugs in the officially recognized as alternative systems of health viz. Ayurveda, Unani, Siddha, Homeopathy and Naturopathy. Phytochemical analysis of natural product extracts used in traditional medicines yield number of constituents and chemical screening gives useful information about the secondary metabolites present.

They exhibit a wide spectrum of activities useful for human race. The chromatographic techniques are used in quantification and identification of phytoconstituents. The secondary metabolites were concentrations in different parts of the plant and are synthesized via various metabolic pathways. These compounds were scientifically proved for the treatment of cancer, antimicrobial agent, antitumor, anti-inflammatory, antioxidant agents, glucosidase inhibitors etc. Based on the structure of natural products many drugs were designed and available in market [1].

Thin Layer Chromatography (TLC)

Thin layer chromatography is a basic, versatile and effective chromatographic technique. It is used to separate mainly non-volatile compounds from the plant materials. The TLC is generally prepared on the glass slide, plastics and aluminum plate which are usually coated with various absorbent materials such as silica gel G or GF, aluminum oxides and cellulose materials.

The particular single or mixture solvent for compound separation was used as the mobile phase and the absorbent materials are used as the stationary phase. The plant extracts were obtained by extraction techniques with different solvents from lower to higher polarity. The extracts were converted to crude extracts by distillation process with appropriate solvents.

The TLC plates were prepared by silica gel-G or Silica gel-GF (It shows Fluorescence under UV radiation). 30 gm of silica gel was weighed accurately and dispersed as a homogenous suspension with 60 ml distilled water for few minutes, then this homogenous suspension was evenly distributed over the TLC plate then plates were subjected to air dry. This TLC plates were dried into a hot air oven at 110°C for 30 minutes and then it stored in a moisture free atmosphere and used it whenever needed. Then the samples were prepared by diluting the crude extracts with a respective solvent or mobile phase.

Then the samples were spotted on the TLC plate (1-10µl volumes) 2 cm above in the plates from the bottom with the help of a capillary tube [2].The movements of the active compounds were expressed by the retention factor (R_f). The R_f values were obtained from the phytochemicals provide the information about the polarity and separation of these phytochemical in the TLC separation process.

Different R_f values of the phytocompounds also give the idea about their polarity by the use of the various solvent systems. The TLC plates were slightly dried and spots were detected with the help of UV light at 254 nm (lower wave length) and 366 nm (higher wave length).

Rf = Distance travelled by solute / Distance travelled by solvent. [3]

High Performance Thin Layer Chromatography (HPTLC)

Herbal drugs which are combination of active constituents. As per the European Pharmacopoeia, herbal drugs are mainly whole or fragmented parts of unprocessed plant parts, typically in dried condition. The quality of herbal drugs and herbal preparations depend on the presence of bioactive constituents and absence of adulteration. Rivaroxaban, an oxazolidinone derivative is first oral bioavailable drug which is a direct inhibitor of factor Xa (Stuart-Prowers factor) with anticoagulant activity. Although many analytical techniques are used for analysis of pharmaceutical dosage form and API, high-performance thin layer chromatography (HPTLC) is used mainly for analysis of plant active constituents.

Automatic TLC Sampler-4 (ATS) has 100 µL syringe and 500 µL syringe with minimum capacity of 100 nL [4].HPTLC has improved resolution and separation than TLC and used for quantitative analysis of the phytoconstituents. Some features include use of higher quality TLC plates with finer particle sizes and spraying techniques to apply microliters of samples as a band. Fig.1 shows the typical scheme of a camag HPTLC instrument.

CAMAG TLC Scanner-4 densitometer

Apart from usual visual detection methods, densitometry gives data used for quantification. They include peak area, peak height. UV, DAD, mass spectrometry (MS), Fourier-transform infrared (FT-IR), and Raman spectroscopy are used for *in-situ* detection of bands on TLC plate [5]. The CAMAG TLC Scanner 4 (densitometer) is the most advanced instrument for evaluation of TLC or HPTLC chromatogram.

The chromatograms are evaluated under visible or UV radiations from 190 to 900 nm. It can measure the reflection either in absorbance or fluorescence mode. Speed of Spectra recording is up to 100 nm/s with steps of 25-200 µm. The densitometer gives visual inspection of electronic images and scanning the whole range to get densitometry [6].

HPTLC is used for the identification of constituents and determination of impurities and quantitative determination of bioactive. It has more accuracy, reproducibility and easy to document the results compared with TLC. The use of High-resolution sorbents which possess selected particle size or chemically modified plates, the versatility of combining with other instruments makes it an ideal TLC technique for the analytical purposes [7].

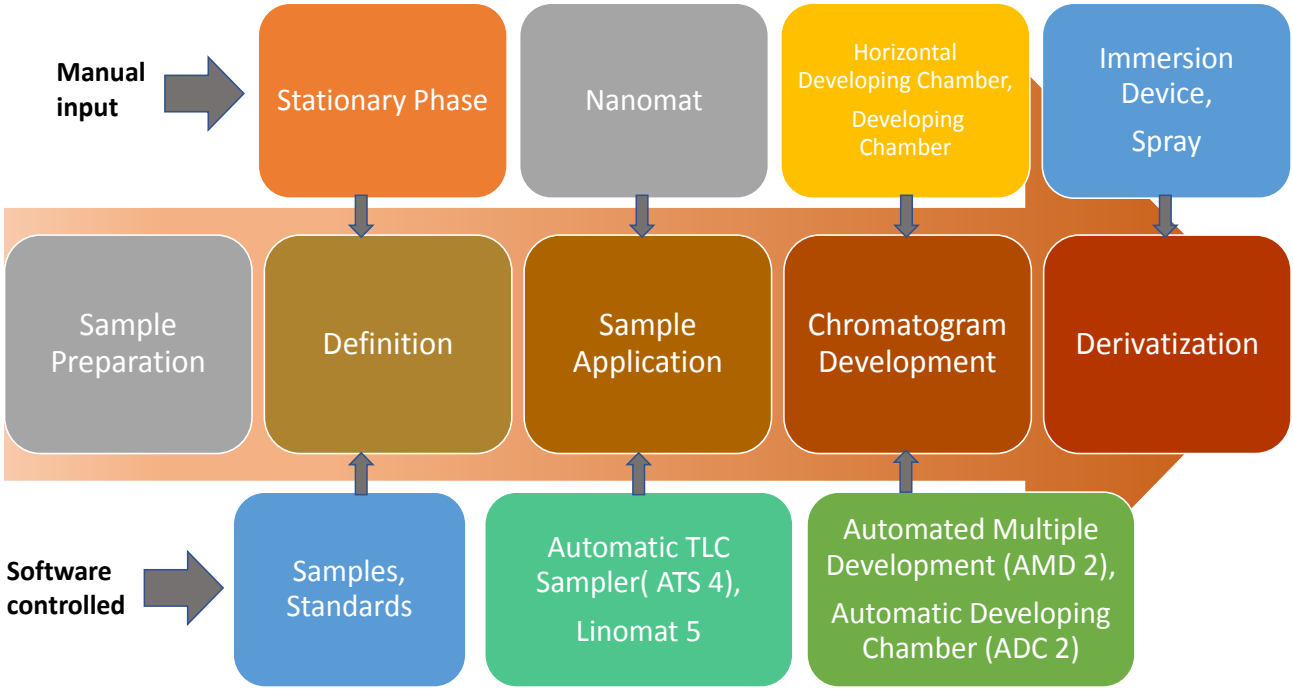

Fig. 1: Flowchart of a typical HPTLC process [8]

Plant Collection and Preparation for Extraction

The plant was selected for the study based on the traditional medicinal use. The plant materials were washed with water to remove foreign particles and kept fresh. Various plants part such as leaves, stem, bark, flowers, fruits, and roots were collected for the study at room temperature for seven days and stored in sterile airtight containers until further use. We should ensure that the plants material was very healthy and uninfected by insects.

The collected plant material specimens should be authenticated by approved Botanist. The collected fresh plant materials were dried in shadow open air. This plant material should not be exposed to sunlight due to loss of potency and volatile matter. Then the dried plant materials were powdered and separated based on the particle size. Then powder was treated with various solvent methods like soxhlet method [9], microwave, ultrasonication, supercritical fluid extraction, solid phase micro extraction etc.

The commonly used solvents with the polarity and secondary metabolites that can be extracted are listed in Table 1. Then it was filtered and the solvent was evaporated using rotary evaporator at a temperature of 45 °C, under reduced pressure. The yield of extractable substances obtained in residual water.

Choice of Solvents

Determination of phytoconstituents from plant materials varies largely with type of solvent used for extraction. Water may be used to extract bioactive with anti-microbial activity compared to alcohols because they have their own anti-microbial activity. Because of methanol's nature to cause blindness to animals in bioassay, it is rarely used for extraction.

Acetone dissolves many hydrophilic and lipophilic components from plant materials and miscible with water, is volatile, and has a low toxicity. Chloroform, dichloromethane, diethyl ether, and ethyl acetate are low polar solvents used to extract low polar constituents.

Petroleum ether, hexane are common non-polar solvents used defatting the plant materials [10].

Table 1: Nature of solvents, its polarity and general uses

S.No	Solvent	Polarity Index	Nature of Polarity	Secondary metabolites can be extracted
1.	Hexane	0.1	Low polarity	Waxes, Fats
2.	Cyclohexane	0.2	Low polarity	Waxes, Fats
3.	Diethyl ether	2.8	Low polarity	Aglycones, Alkaloids
4.	Dichloromethane	3.1	Low polarity	Terpenoids, Flavonoids, Aglycones
5.	Chloroform	4.1	Low polarity	Terpenoids, Flavonoids, Alkaloids, Aglycones
6.	Ethyl acetate	4.4	Low polarity	Aglycones, Alkaloids, Glycosides
7.	Acetone	5.1	Medium Polarity	Flavonols, Alkaloids, Aglycones.
8.	Ethanol	5.2	Medium Polarity	Tannins, Polyphenols, Flavanols, Terpenoids, Sterols, Alkaloids, Polyacetylenes, Propolis,
9.	Methanol	6.6	Medium Polarity	Saponins, Tannins, Phenones, Flavones, sugars, Lectins, Terpenoids, Anthocyanins, Starches and Polypeptides, Aminoacids, Xanthoxyllines, Totrol, Quassinoids, Lactones and Polyphenols.
10.	Water	9.0	High polarity	Sugars, Aminoacids, Saponins, Tannins, Lectins, Terpenoids, Anthocyanins, Starches and Polypeptides.

Qualitative Chemical Tests for the Secondary Metabolites

It is important to confirm the presence of type of constituents before running the chromatography so as to choose the appropriate solvent system. The following tests give idea about the type of constituents present in the extract under research.

Test for Alkaloids

- **Mayer's Test**

To a few ml of herbal extract, add two drops of Mayer's reagent (a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water) through the sides of test tube. Formation of white creamy precipitate shows the presence of alkaloids [11].

- **Wagner's Test**

To a few drops of Wagner's reagent (2.5 gm I_2 is dissolve in 12.5 gm of KI add 250 ml of water to produce solution) are added to the few ml of plant extract through the sides of test tube.

A reddish- Brown precipitate was appeared which indicates the presence of alkaloids in the plant extraction[11].

- **Dragendorff's Test**

To a few ml of crude extract is added to 1% HCl, steam it for 10 minutes. To this add 6 drops of Dragendorff's reagent (a solution of potassium bismuth iodide). Reddish brown precipitate indicates the presence of alkaloids [12], [13].

- **Hager's Test**

The extract give yellow color precipitate with Hager's reagent [saturated Picric acid in water] if alkaloids are present [14].

- **Tannic Acid Test**

The extract give buff color precipitate with 10% Tannic acid solution if alkaloids are present.[14]

Test for Amino Acids

The plant extract (0.1gm) was dissolved in distilled water and filtered through Whatmann filter paper No. 1 and the filtrate was test for Amino acids [11].

Ninhydrin Test

A two drops of ninhydrin (IUPAC name: 2,2-dihydroxyindane-1,3-dione) solution (0.1 gm of ninhydrin in 200 ml of an acetone) are added to 2 ml of the aqueous filtrate. The purple colour was Appeared which indicates the presence of Amino acids in the medicinal plant extraction.

Test for Carbohydrates [14]

- **Benedict's Test**

To a few ml of filtrate, 0.5 ml of Benedict's reagent (100 g of anhydrous sodium carbonate, 173 g of sodium citrate and 17.3 g of copper (II) sulfate pentahydrate) is added through the sides of test tube. The extraction and reagent mixture is heated on a boiling water bath for 2 minutes. The colored precipitate indicates the presence of sugar moiety.

- **Caramelisation**

Extract is treated with strong sulfuric acid, if they undergo charring with the dehydration along with burning sugar smell it indicates presence of carbohydrates.

- **Fehling's Test**

Fehling's A (Copper sulfate in distilled water) and Fehling's B (Potassium tartrate and Sodium hydroxide in distilled water) reagents are mixed in equal volume. To this solution few drops of extract is added and boiled. Formation of brick red precipitate of cuprous oxide forms, if reducing sugars are present.

- **Molisch' s Test** [14]

To two ml of the herbal extract, two drops of a alcoholic solution of Molisch reagent (Dissolve 3.75 g of 1-naphthol in 25 ml of Ethanol 99%) are added. To the plant extract add few drops of conc. sulfuric acid along the sides of test tube. The violet ring was appeared which indicates the presence of carbohydrates in the extract.

- **Seliwanoff's Test**

The Seliwanoff's reagent (resorcinol and concentrated HCl). Conc. HCl reacts with keto-sugar in extract to form furfuraldehyde derivative, it gives red color when coupled with resorcinol. Fructose gives red color soon [14].

Test for Fixed oil and Fat [12]

- **Spot Test**

A spot of herbal extract is pressed between folds of filter paper, the oily stain on the filter paper indicates the presence of fixed oils in the plant extract.

- **Saponification Test**

A few drops of 0.5 N alcoholic KOH solution is added to a small quantity of herbal plant extract along with a drop of phenolphthalein indicator then the mixture is heated for two hours on a water bath. The development of soapy solution shows the positive test.

Test for Glycosides

For 0.05gm of medicinal plant extract is hydrolyzed with concentrated HCl. The mixture is heated for 2 hours on water bath. Then the filtrate is used for the furthur tests.

- **Borntrager's Test**

To two ml of a filtrate, add 3 ml of chloroform and shaken well, 10% ammonia solution is added to chloroform layer. The Pink colour shows the presence of anthraquinone glycosides in the plant extract [11].

- **Bromine water Test**

When the test solution is treated with bromine water it gives yellow precipitate which shows presence of glycosides [14].

- **Legal's Test**

To test solution in pyridine sodium nitroprusside solution is added and 10% NaOH was added. The Pink color shows positive for glycosides in the plant extract [11].

- **Keller-Killiani Test**

The test solution was mixed with 2ml of glacial acetic acid and few drops of 2% $FeCl_3$.

Then treated with 2ml of conc. H_2SO_4. Appearance of brown ring at the interphase indicates the presence of cardiac glycosides.[14]

- **Raymond's Test**

Test solution is treated with dinitro-benzene in hot methanolic alkali produces violet color indicates the presence of glycosides [14].

Test for Phenolic Compounds

Ferric Chloride Test

The plant extract is dissolved in of distilled water. To this add a few drops of neutral 5% $FeCl_3$ solution. The dark green color was appeared which indicates the presence of phenolic compound in the plant extract.

- **Gelatin Test**

The plant extract is dissolved in distilled water and 1% solution of gelatin containing 10% NaCl. The white precipitate was appeared which indicates the presence of phenolic compounds.

- **Lead acetate Test**

The plant extract is dissolved in distilled water and 3 ml of 10% lead acetate solution is added to it. The bulky white precipitate was appeared which indicates the presence of phenolic compounds in the plant extract [11].

Test for Tannins

Braemer's Test

To 2-3ml of the extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey color indicates the presence of tannins.

Mitchell's Test

With iron and ammonium citrate or iron and sodium tartrate. Tannins give a water-soluble iron-tannin complex, which is insoluble in solution of Ammonium acetate.

Vanillin Hydrochloride Test

Test solution was treated with few drops of vanillin hydrochloride reagent gives purplish red color.

Test for flavonoids

Alkaline Reagent Test

An aqueous extract is treated with 10% NH_4OH solution. The Yellow fluorescence was appeared which indicates the presence of flavonoids in the plant extraction [14].

Shinoda Test

A few magnesium ribbon fragments and conc. HCl were added to the ethanolic extract. The appearance of pink scarlet, crimson red or green to blue color after few minutes which indicates the presence of flavonoids in the plant extract.

Zinc Hydrochloride Reduction Test

To the test solution add zinc dust and conc. HCl. It gives red color after few minutes which indicate the presence of flavonoids.

Test for Phytosterols

- **Libermann-Burchard's Test**

To the extract, 1ml of chloroform, 2-3ml of acetic anhydride and 2-3 drops of conc. sulphuric acid were added. Appearance of green color indicates the presence of steroids and the presence of terpenoids is indicated by dark pink or red coloration of the solution [12].

Test for Proteins

The plant extract is dissolved in distilled water and filtered and the filtrate is used for following tests.

- **Millon's Test**

To 2 ml of filtrate few drops of Million's reagent (dissolving metallic mercury in nitrous acid and diluting with water) are added. A white precipitate indicates the presence of proteins.

- **Biuret Test (Piotrowski's Test)**

2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution and ethanol (95%), followed by excess of potassium hydroxide pellets. Pink color indicates the presence of protein [11].

Test for Saponins

- **Foam Test**

The plant extract is dispersed in distilled water and made up to 20 ml and made into suspension. The foam was appeared which indicates the presence of saponins in the plant extract [11].

Test for Gum and Mucilage

The plant extract is dissolved in 10 ml of distilled water and added 2 ml of absolute alcohol is added with stirring. The white or cloudy precipitate indicates the presence of Gums and Mucilage [11].

Test for Volatile Oil

A 50 mg of powdered plant material is taken and do hydro-distillation. The distillate of the extraction is collected in graduate tube; we can separate the aqueous portion from the volatile oil [11].

TLC Solvent System

The selection of solvent system is perhaps the most important parameter to achieve efficient separation in TLC. And this might be the challenging part of the study. If starting a new unknown separation start with a 50% Ethyl-acetate/Hexane, if it's not working try Methanol / Dichloromethane (DCM) at last try toluene with acetone, Ethyl-acetate, or DCM. Feel free to change the ratio of every system to optimize the system because varying the ratio shows marked effect on the R_f. Table 2 documents the solvent system and R_f values of some therapeutically important herbals.

Table 2: TLC solvent system for plant constituents with R_f values and used

S.No	Plant Name	Parts used	Constituents	Solvent system	Spot Identification method	R_f Value	Uses	References
1.	*Gentiana lutea*	Roots	gentiopicroside	Ethyl acetate/methanol/water (77:15:8 v/v/v)	detected by UV 254 nm	0.58	Appetite-stimulating drug, hepatoprotective	[15], [16]
2.	*Elaeocarpus sphaericus*	Fruits	Rudrakin	methanolic extract on silica gel G. n-butanol: acetic acid: water (4:1:5)	detected by UV 366 nm shows violet fluorescence	0.91	Anticonvulsant and analgesic	[17]
3.	*Rauwolfia serpentina*	Root and callus	Reserpine	Methanolic extract, Chloroform: methanol (97:3, *v/v*)	Spraying Dragendorff 's reagent develops orange spots	0.46 to 0.48	Antihypertensive agent	[18]
4.	*Reinwardtia indica*	Leaves	tannin	Methanolic extract Chloroform: Water (6:4, v/v)	FeCl3 spray	0.85	Treatment of paralysis and as natural antibiotic.	[19]
5.	*Trigonella foenum-graecum*	Seed	Trigonelline	Methanolic extract. n-propanol:methanol:water (4:1:2, v/v/v)	Detected by UV 269 nm.	0.46	Anti-diabetic, Antibacterial, Gastric stimulant.	[20]
6.	*Holarrhena antidysenterica*	Stem Bark	Conessine	Methanolic extract, Toluene: ethyl acetate: diethyl amine (6.5:2.5:1, v/v/v)	derivatized the plate with modified *Dragendroff's* reagent.	0.82	Amoebic dysentery and diarrhoea	[21]
7.	Althaea officinalis	Flowers and leaves	flavonoids	Chloroform: methanol (80:20, v/v)	Detected by UV 254nm.	0.45	Antimicrobial, to treat digestive, respiratory, and skin conditions	[22], [23]
8.	*Holarrhena antidysenterica*	Dry bark	Conessine	Petroleum ether: chloroform (85:15, v/v)	Sprayed with Dragendorff's reagent gives intense orange spot	0.82	Treat to diarrhoea and amoebic dysentery	[24]
9.	*Phyllanthus*	fruit powde	Gallic acid	Methanolic extract,	Detected by	0.40	Antimicrobial, antioxidant, anti-	

	emblica	r		toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2, v/v/v)	UV 278 nm.		inflammatory, analgesic and antipyretic properties.	[25]
10.	*Jatropha curcas*	Root	Alkaloids	Methanolic extract, Benzene: chloroform: few drop acetic acid (9:1, v/v)	Mayer's reagent	0.81, 0.66, 0.63, 0.49	Antibacterial activity	[26]
11.	*Clitoria ternatea*	Root	taraxerol	Hexane: ethyl acetate (80:20, v/v)	anisaldehyde reagent.	0.53	Antioxidant, anticancer	[27]
12.	*Asparagus racemosus*	Roots	Alkaloids, Flavonois, Tannins, phenolics	Chloroform: methanol (3:7, v/v)	Detected by UV 254nm.	0.4, 0.45 and 0.48.	Antimicrobial activity	[28]
13.	*Trigonella foenum graecum*	Fenugreek seeds	trigonelline	n propanol: methanol: water (4:1:2, v/v/v)	detected by UV 254	0.43	Anti ulcer, wound healing, immunomodulatory, CNS stimulant.	[29]
14.	*Wedelia chinensis*	leaf material	Alkaloid, Flavonoid, Tannins	Chloroform: Methanol (12:2, v/v), Ethyl acetate: Butanol: Formic acid (2.5:1.5:0.5, v/v/v) Methanol :Water (6:4, v/v)	Dragendorff reagent (Orange) AlCl3 reagent (Orange), FeCl3 reagent (Brownish Grey)	0.8, 0.87, and 0.83	Treating viral hepatitis, Fever and infection.	[30]
15.	*Wedelia chinensis*	leaf	Phenols	Chloroform: Methanol (27:0.3, v/v) Ethyl acetate: Toluene: Formic acid (2.2:1.1:1.1, v/v/v)	Folin Ciocalteau reagent (Blue). FeCl3 reagent (Green)	0.87 And 0.8	Treatment of bites and stings,	[30]
16.	*Anethum graveolens*	Seeds	Alkaloid, Cardiac glycosides	ethyl acetate: methanol: water (100:13.5:10, v/v/v)	Visible and detected by UV 254 nm	0.226, 0.441	Carminative, stomachic and diuretic	[31]
17.	*Foeniculum vulgare*	Seeds	Flavonois and Alkaloid	ethyl acetate: methanol: water (100:13.5:10, v/v/v)	Visible	(0.243, 0.328, 0.414, 0.757, 0.786) and 0.226	To treat gastrointestinal disorders	[31]
18.	*Trachyspermum ammi*	Seeds	Cardiac glycosides and Tannins	ethyl acetate: methanol: water (100:13.5:10, v/v/v)	Visible	0.794 And 0.296	To treat asthma and amenorrhoea	[31]
19.	*Aegiceras corniculatum*	leaves	Gallic acid	TLC Silica gel $60F_{254}$, Hexane: ethyl acetate (1:9, v/v)	Derivatization of TLC plates was done and UV light at 254nm.	0.25	Anti-tuberculosis, antibacterial and antioxidant activities	[32]
20.	*Atropa belladonna*	dry roots	Atropine	chloroform: methanol (80:20,v/v)	detected by UV 254	0.74	Reduce salivation and bronchial secretions before surgery	[33]

21.	*Aloe vera*	matur e leaves	Barbaloin	precoated Kieselger 60 plates Chloroform-95%: ethanol: H2O (60:30:2, v/v/v).	spraying 0.1 M borax solution (in 50% methanol aqueous solution) excitation wavelength, 383 nm; emission wavelength, 550 nm	0.52	Heals burns. Improves digestive health Clears acne	[34], [35],
22.	*Glycyrrhiza glabra*	rhizom es	Glycyrrhizin	RP-18 silica gel 60 F254S Methanol: water (7: 3, v/v)	detected by UV 256 nm	0.63	treatment of hypoglycemia, treatment of dropsy	[36]
23.	*Andrographis paniculata*	dried powde r	Andrographolide	silica gel 60 F254 chloroform: methanol (9:1, v/v)	detected by UV 250 nm, detected by UV 228 nm	0.39	treatment of cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases	[37]
24.	*Curcuma longa*	rhizom es	Curcuminoids, curcumin, Demethoxy curcumin, Bisdemethoxycurcumin	chloroform: benzene: methanol (80:15:5, v/v/v)	detected by UV 366	0.69, 0.51, 0.39	treatment of inflammation, infectious diseases, and gastric, hepatic, and blood disorders	[38]
25.	*Phyllanthus niruri*	roots, leaves, fruits	Phyllanthin, gallic acid	silica gel 60F254 toluene: ethyl acetate: formic acid (5: 3.5: 0.5, v/v/v)	detected by UV 254 nm	0.3 , 0.63	treatment of jaundice, gonorrhoea, frequent menstruation, and diabetes	[39]
26.	*Allium cepa*	Bulbs	Quercetin, Quercetin-4'-O-glucoside, Quercetin-3-O-rhamnoside	RP-18 - silica gel Tetrahydrofuran: water: formic acid (40:60:6, v/v/v)	detected by UV 366 nm	0.17, 0.30, 48.2	antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-diabetic, hypolipidemic, anti-hypertensive	[40]
27.	*Tephrosia purpurea*	Leaves	rutin, quercetin	silica-gel RP-18 F 254S methanol: water: formic acid (40:57:3, v/v/v)	detected by UV 254 nm	0.17 0.07	antibacterial property, diuretic, cyto-toxicity and diabetics	[41]
28.	*Terminalia arjuna*	bark	gallic acid	toluene: acetic acid: ethyl acetate (1:0.1:1, v/v/v)	detected by UV 254 nm	0.5	antioxidant, anti-inflammatory, antimicrobial, anti-hypertensivel, anginal pain, hypertension, congestive heart failure, and dyslipidemia	[42]
29.	*Strychnos nux-vomica*	seeds	Strychnine, brucine	HPTLC: toluene: ethyl acetate: diethyl amine: methanol (7:2:1:0.3, v/v/v/v)	detected by UV 254 nm	Strychnine (0.55) and brucine	Male infertility and impotence, menstrual problems, migraine, constipation.	[43]

						(0.43)		
30.	*Catharanthus roseus*	leaves	Quercetin, rutin	Ethanolic extract, Pre coated silica gel 60F254 Toluene: Ethyl Acetate: methanol (5:3:2, v/v/v)	Densitometric determination and quantification of these compounds was carried out at 254 nm	rutin and quercetin are 0.17 and 0.65	Antioxidant, anticancer	[44]
31.	*Ficus fiskei*	leaves	gallic acid, ellagic acid, quercetin, rutin	toluene: ethyl acetate: methanol (6:3:2, v/v/v)	Shinoda's test Sodium Hydroxide test-Red color, Yellow color	Endemic Philippines Ficus fiskei Elm		[45]
32.	Madhuca indica	inner bark	Alkaloids, Corbohydrates, Glycosides, Phenolic compounds	Methanol extract Hexane: Acetic acid (9:1, v/v)	Visible	0.36, 0.73, and 0.96	Antidiabetic, antiulcer	[46]
33.	Piper betle	leaves	Allylpyrocatechol	ethanol extract precoated silica gel plates ethyl acetate: n-hexane (7:3, v/v)	sprayed with vanillin-sulfuric acid, Visual, UV-254 nm, UV-366 nm	0.86, 0.13	antibacterial, antifungal, antioxidant, cytotoxic, antihelminthic, antiprotozoal, antidiabetic, hepatoprotective, and immunomodulatory properties	[47]
34.	Cosciniu m fenestrat um	Stem	berberine	Precoated silica gel GF 254 plates butanol: glacial acetic acid: water (14:3:4, v/v/v)	detected by UV 254 nm	0.55	a bitter tonic, used in dressing wounds, treating ulcers	[48]
35.	Himanthalia elongata	seaweed	fucoxanthin	Chloroform: diethyl ether: n-hexane: acetic acid (10:3:1:1, v/v/v/v)	visible	0.40	antioxidant and antimicrobial	[49]
36.	Piper betle	grinded leaves	Eugenol	Methanolic extract toluene :ethyl acetate (93:7, v/v)	Detected by 254 nm and 366 nm, scanned under UV light	0.60	antifungal-activity	[47]
37.	Santalum album	wood	a-santalol, l-linalool, diosgenin	cyclohexane: ethyl acetate (9:1, v/v)	ethanolic solution of p-anisaldehyde (25%), sulphuric acid (35%) and glacial acetic acid (16%), specific for sesquiterpe	0.988, 0.932, 0.988	Antibacterial, antiseptic, antipyretic	[50]

					noid			
38.	Callistemon citrinus	fresh leaves	1,8-cineole	Toluene: Ethyl acetate: Methanol: Formic acid (9:0.5:0.5:0.5, v/v/v/v)	TLC plate was visualized after derivatization with anisaldehyde sulfuric acid reagent at 540 nm.	0.52	hepatoprotective, cytotoxic, cardio-protective, anti-Helicobacter pylori, and anti-bacterial activity	[51]
39.	Myristica fragrans	bulb	Myristicin, Eugenol	Benzene: Toluene: Ethyl acetate (93:7, v/v)	Scanned in 299 nm and 279nm	0.81, 0.66	to treat stomach ulcers, indigestion, liver disorders	[52]
40.	Citrus X sinensis	Peels	hesperidin	Methanol: Chloroform (4:6, v/v)	Detected by UV 278 nm	0.61	Treat for scurvy	[52]
41.	Aloe vera	Bulb	Aloin, aloe-emodin	Ethyl acetate: methanol: water (10:2:1, v/v/v)	Detected by wavelength of 254 and 366 nm respectively	0.80, 0.83	Anti-plasmodial potential	[53]
42.	Apium graveolens	Leaves	Limonene, Apigenin	Chloroform: n-Butanol: Acetic acid: water (4:1:5, v/v/v)	Spray reagent - Vanillin Sulphuric acid, Spray reagent, Iodine Vapour	0.46, 0.58	aphordisiac, anthelmintic, antispasmodic, laxative, emmenagogue,	[54]
43.	Hypericum perforatum	Flowering aerial parts	Hypericin	Methanol extract Chloroform: Methanol (8:2, v/v)	sprayed with a 0.2%DPPH solution in MeOH - yellow spots against a purple background spectrophotometer at 517 nm	0.27	Antiradical activity, anti viral activity	[55]
44.	Myricana gi Thunb	fruit	gallic acid, quercetin, myricetin, caffeic acid	Methanol extract ethyl formate: toluene: formic acid: water (20:1:2.6:0.5, v/v/v/v)	UV detection at 254 nm	0.57, 0.98, 0.53	Asthma, cough, chronic bronchitis, ulcers, inflammation, anaemia, fever, diarrhoea, and ear, nose, and throat disorders.	[56]
45.	Syzygium cumini	Seeds and bark	Elagic acid, Galotannin, 3-galloyl glucose, Corilagin	n-butnol:acetic acid:water (4:1:5, v/v/v)	All spots gave blue colour with KFe(CN),-$FeCl_3$, dip reagent and brown to reddish brown colour with ammoniacal silver	0.35, 0.19, 0.18 And 0.29	the treatment of sore throat, bronchitis, asthma,	[57]

					nitrate.			
46.	Hiptage benghalensis	Aerial part	Quercetin, Kaempferol	Benzene: Acetic Acid: Water (125:72:3, v/v/v)	The developed plates were also sprayed with 5% FeCl3, 0.1% alcoholic AlCl3 for further confirmation.	0.78, 0.95	burning sensation, wounds, ulcers, inflammations and leprosy	[58]
47.	Alhagi maurorum	Stem, Leaves and Flower	luteolin	conc. HCl: Acetic acid: Water (3:30:10, v/v/v)	sprayed with 5% FeCl3	0.6	purgative, diaphoretic, expectorant	[59]
48.	Cannabis sativa	leaves, stem and seeds	cannabinol R	Heptane:dichloromethane:butan-2-one (83:5:12, v/v/v)	Dragendoff's reagent-orange colored spots	0.50	Hallucinogenic relax	[60]
49.	Catharanthus roseus	Flower, stem	vincristine	Ethy1 acetate: absolute alcohol (3:l, v/v)	Dragendorff's reagent	0.18	Anticancer agent	[61]

Conclusion

This review reveals the potential of the thin-layer chromatography procedure used for investigation of the phytoconstituents from the plant materials. The TLC is the essential tool to identify and quantify the phytoconstituents present in the plant materials by many researchers. This review article explains the plant collection, plant extraction, phytoconstituents analysis, TLC studies, HPTLC studies and solvent applications. These TLC procedures in this article will be useful for the plethora of researchers in phytoconstituents research.

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