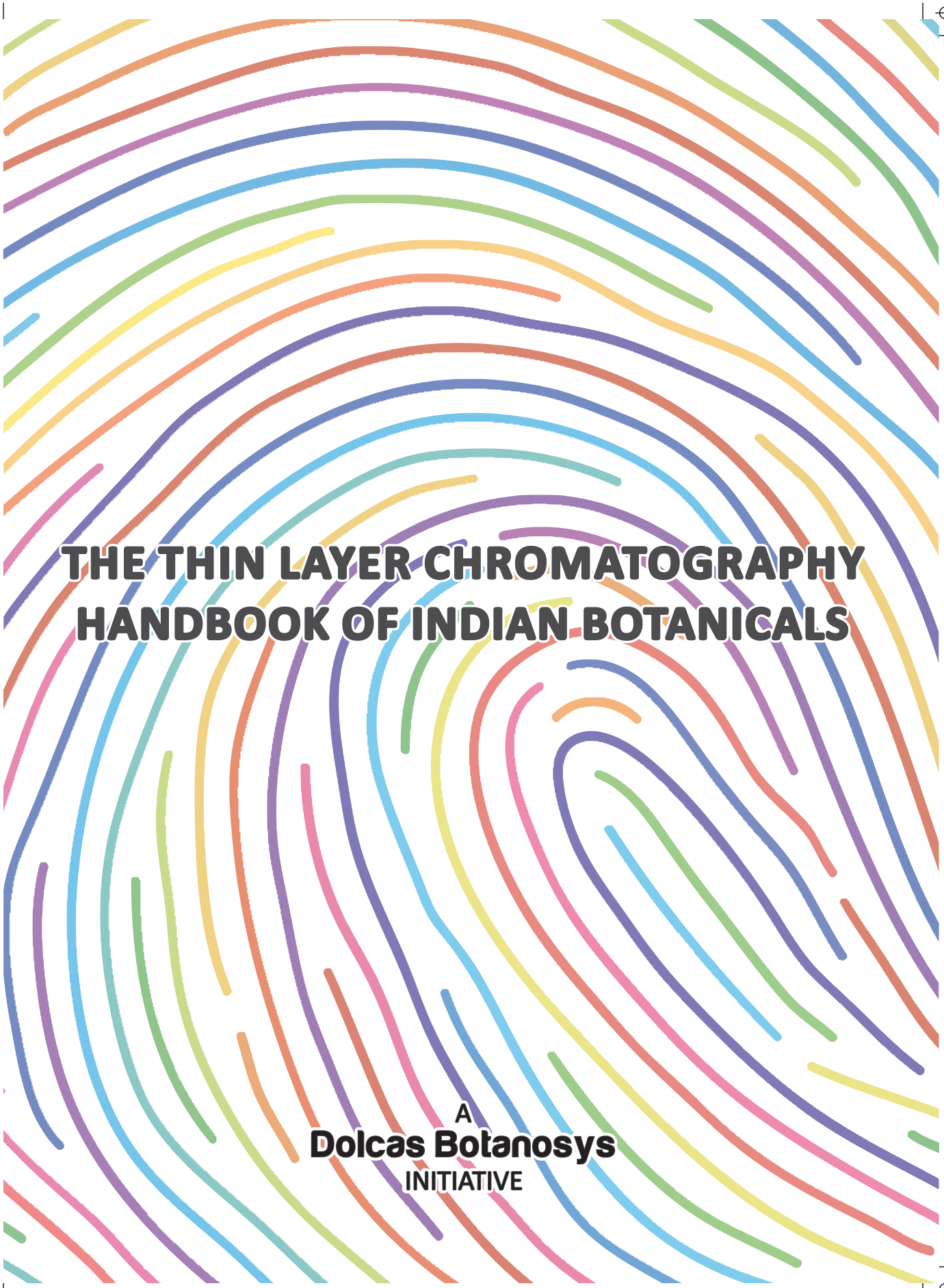

THE THIN LAYER CHROMATOGRAPHY HANDBOOK OF INDIAN BOTANICALS

A

Dolcas Botanosys

INITIATIVE

The Thin Layer Chromatography Handbook of Indian Botanicals

A **Dolcas Botanosys** Initiative

Dr. Swati Joshi, Sampath M, Zakir Hussain, Dimple Kumari, Sanyam Verma

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FOREWORD

Dear Friends, Patrons and Collegues

As we usher into the New Year pursuing exciting new opportunities in an increasing worldwide acceptability of Natural Medicine, we also face an unprecedented on us of accountability for our products. The elevated awareness for healthy living and goodness of herbs has brought about hundreds of new products that are targeted for new health application and manufactured by new technologies. Consequently, there is also a spurt in the demand for scientific validation of herbal and natrual medicine.

While on one hand, the industry struggles with complex issues such as heavy metal toxicity and bioavailability, on the other elementary issues like identification remain clouded in controversy. And rightly so, because the sheer complexity of herbal drugs allows unscrupulous manufacturers to pass of adulterated or unauthentic herbs in the name of genuine products. The direct loser, naturally, is the consumer. But that's not all, it also dents the repute of the herbal industry as a whole.

Dolcas Botanosys has always endeavored to educate its customers, bringing transparency in our operations and initiating dialogue by way of our newsletter, BotanoSpeak. This Handbook is another such initiative.

Catalogued here are the TLC Profiles of over 40 common Indian herbs. The references are taken from National Pharmacopoeias for most, and if not available, from acknowledged publications of repute to impart reliability and reproducibility to the work. We encourage the reader to put these into practice in their QC or R&D Departments. The coloured images of the herb TLC parallel to its extract TLC will helps visualize and compare the outcomes.

TLC authentication of a raw herb or extract is in fact the essential first step in Quality conformance for an herbal products business and we sincerely hope this manual will prove useful in that direction.

With our best wishes for a health and success in the New Year!

Sincerely

Rtvik Sethia
Managing Director

Introduction

A medicinal plant is any plant which, in one or more of its parts, contains substances that can be used for therapeutic purpose or which is a precursor for synthesis of drugs. Any plant that possesses therapeutic properties or exerts beneficial pharmacological effects on an animal system is also designated as medicinal plant. According to WHO 80% of the population in the developing countries depend upon traditional herbal medicine.

Therapeutic properties of medicinal plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Nowadays people are being targeted with thousands of unhealthy products, the level of Sensitivity to diseases is very high and here the medicinal plants play a pivotal role as an alternative for synthetic products. Many plants synthesize chemical substances called phytochemicals like phenolics, alkaloids, amino acids, flavonoids, saponins and glycosides that are useful to the maintenance of health in humans and other animals.

In recent years, there has been a wide interest among researchers for identification of medicinal potent phytochemicals from natural sources. There are several chromatographic techniques are available for identification of various Phytochemicals. Chromatographic such as Paper chromatography, Thin Layer chromatography (TLC), High Performance Thin layer Chromatography (HPTLC) and Electrophoresis (CE) have been routinely used to identify the plant derived chemicals.

Although there are many chromatographic techniques, TLC is very important. TLC does not require complex instrumental setup, plates are disposable, so there is no memory effect and furthermore, it is an easy, inexpensive method that can be used in any laboratory. In natural and cosmetic industries TLC has been frequently used for the separation and semi-quantitative analysis purpose. It can be used to determine the number of components present in a mixture, the identity of compounds, and their purity. It can also be used to control isolation of compound in column chromatography by identifying the individual elutes.

Many of the herbal based pharmaceutical companies are manufacturing the various herbal ingredient or phytochemicals. Few among them are resorting to unethical practices like adulteration. These adulterant molecules mostly have same physical organoleptic properties with original herbal ingredient or phytochemicals.

It is very difficult and challenging to find out those adulterant in industrial quality control sectors. Generally, pure standards of many phytochemicals are not available in market due to its high cost and complexity of its nature and purification strategy. In this study an attempt has been made to establish a correlation between the herb TLC and the TLC of its extract to conclude its authenticity.

Thin layer chromatography

Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC consists of three steps - spotting, development, and visualization.

Spotting

Spotting consists of using a micro pipette to transfer a small amount of this dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic sheet. The spotting solvent quickly evaporates and leaves behind a small spot of the material.

Development

Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent or mobile phase, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the adsorbent (silica gel plate) and the development solvent for the spotted material. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate.

Visualization

Visualization of colored compounds is simple – the spots can be directly observed after development. Because most compounds are colorless however, a visualization method is needed. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light. A spot will interfere with the fluorescence and appear as a dark spot on a glowing background. While under the UV light, the spots can be outlined with a pencil to mark their locations. A second method of visualization is accomplished by placing the plate into iodine vapors for a few minutes. Most organic compounds will form a dark-colored complex with iodine. It is good practice to use at least two visualization techniques in case a compound does not show up with one particular method.

R_f calculation

The Rf value is used to quantify the movement of the materials along the plate. By using this Rf value we can compare or make tentative identification of chemical compounds. Generally, Rf is equal to the distance traveled by the substance divided by the distance travelled by the solvent. Its value is always should be less than 1.

$$R_f = \frac{\text{Distance travelled by the substance (Y)}}{\text{Distance travelled by the Solvent (X)}}$$

Effect of adsorbent

Two most common adsorbent coatings for thin-layer chromatography plates are Alumina (Al_2O_3) and Silica Gel (SiO_2). Alumina, when anhydrous, is the more active, that is, it will adsorb substances more strongly. It is the adsorbent of choice when the separation involves relatively nonpolar analytes such as hydrocarbons, alkyl halides, ethers, aldehydes, and ketones. To separate the more polar substrates such as alcohols, carboxylic acids, and amines, the less active adsorbent, Silica Gel, is used. In extreme situations, very polar substances on Aluminum Oxide do not migrate very far from the starting point and nonpolar compounds travel with the solvent front if chromatographed on Silica Gel.

Effect of mobile phase Solvent

A polar solvent will carry along with it polar analytes and nonpolar solvents will do the same with analytes that are themselves nonpolar. Common solvents used in chromatography, both thin-layer and column, are listed below. The higher a solvent's Dielectric Constant the more polar it is.

Solvent	Dielectric Constant	Solvent	Dielectric Constant
Hexane	1.9	Ethyl Acetate	6.0
Petroleum Ether	2.0	Acetic Acid	6.2
Cyclohexane	2.0	Isopropyl Alcohol	18.3
Carbon Tetrachloride	2.2	Acetone	20.7
Benzene	2.3	Ethanol	24.3
Toluene	2.4	Methanol	32.6
Diethyl Ether	3.4	Water	78.5
Chloroform	4.8		

Effect of Sample spot

Resolution is a measure of how well the analyte spots separate from each other. Certainly, for good resolution, the analytes should have distinct Rf values. However, the Breadth of the spot also affects the chromatogram's resolution. As each compound migrates, its spot broadens as material diffuses away from its center. The reasons for this broadening are complex and will not be discussed here. Note only that the longer the system is allowed to develop, the broader will be the spots. Good resolution requires that each spot have minimal breadth. Selecting a chromatographic system, adsorbant and eluent, such that all the analytes have distinct Rf's and minimal spot broadening can be tricky.

PREPARATION OF SPRAY REAGENTS

1. Anisaldehyde-Sulphuric acid reagent

0.5 ml Anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid.

2. Dragendorff reagent

Solution (a): Dissolve 0.8 g basic bismuth nitrate in 10 ml glacial acetic acid and 40 ml water under heating. If necessary, filter.

Solution (b): Dissolve 8g potassium iodide in 30 ml water.

Stock solution: (a) + (b) are mixed 1:1

Spray agent: 1 ml stock solution is mixed with 2ml glacial acetic acid and 10 ml water.

3. Iron-III- chloride reagent ($FeCl_3$)

Dissolve 1 gm of $FeCl_3$ in 100m l of water.

4. Sulphuric acid reagent (H_2SO_4)

Ethanolic sulphuric acid as per the strength mentioned in the monograph (10-20%). The plate is heated at 100°C for 3-5 min, evaluation in visible light.

Concentrated H_2SO_4 colored zones appear immediately

5. Vanillin boric acid reagent

Dissolve 0.5 g vanillin and 5g of boric acid are dissolved in10 ml of H2SO4 and further dissolved in 500 ml of ethanol.

6. Vanillin-sulphuric acid reagent

1% ethanolic vanillin (Solution I)

Dissolve 1gm of vanillin in 100ml of Ethanol

10% ethanolic sulphuric acid (Solution II)

Dissolve 10 ml of sulphuric acid in 90ml of Ethanol

The plate is sprayed with 10 ml solution I, followed immediately by 10 ml solution II. After heating at 110°C for 5-10 minutes under observation, the plate is evaluated in normal light.

7. Iodine Chamber

Take a 250 ml clean and dry beaker and charge beaker with some iodine crystal now place the developed chromatogram into iodine vapour. Generally spot will appear brown/orange in colour.

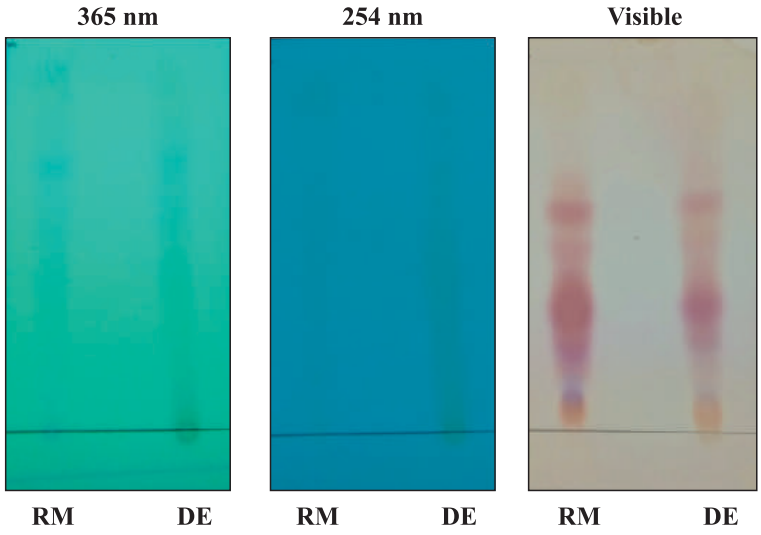
Note: Iodine detection is relatively unspecific, universal detection method for many phyto molecule.

Allium sativum

Other Names : Lahsun, Garlic

Marker Compounds : Alliin, Allicin, S-allylcystine,

Plant Part Used : Bulb



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : n-butanol: n-propyl alcohol: Glacial acetic acid: water (3:1:1:1v/v/v/v)

Spray Reagent : 0.2% (w/v) of Ninhydrin in 95 volume of n-butanol and 5 vol of 2 M acetic acid

Inference : Blue spot R_f- 0.12; Pink spot R_f- 0.35; Pink spot R_f- 0.52; Pink spot R_f- 0.63

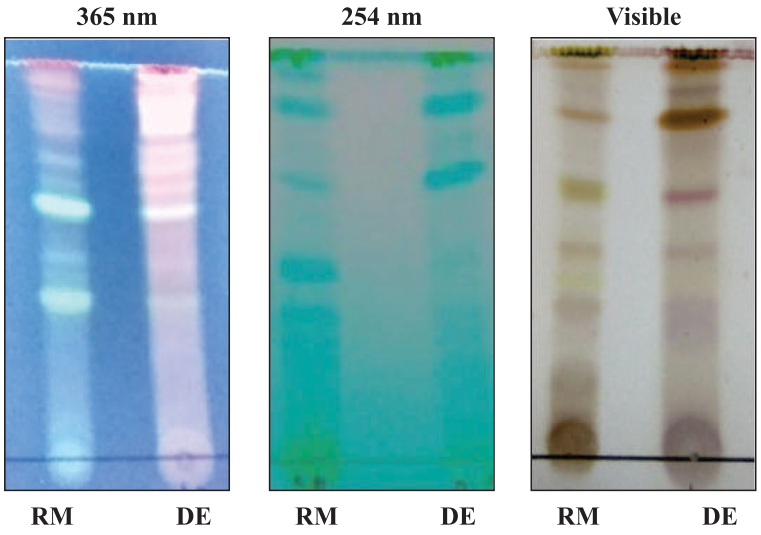
Reference : Indian Pharmacopeia 2010; P 2517-2518.

Andrographis paniculata

Other Names : Kalmegh, Indian Echnaecia

Marker Compounds : Andrographolides, neo-andrographolide Andrographanin

Plant Part Used : Whole Plant



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol.

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform: Methanol (9:3 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Pink R_f -0.33; Green R_f -0.61; Pink R_f -0.81; Pink R_f -0.89; Brown R_f -0.94

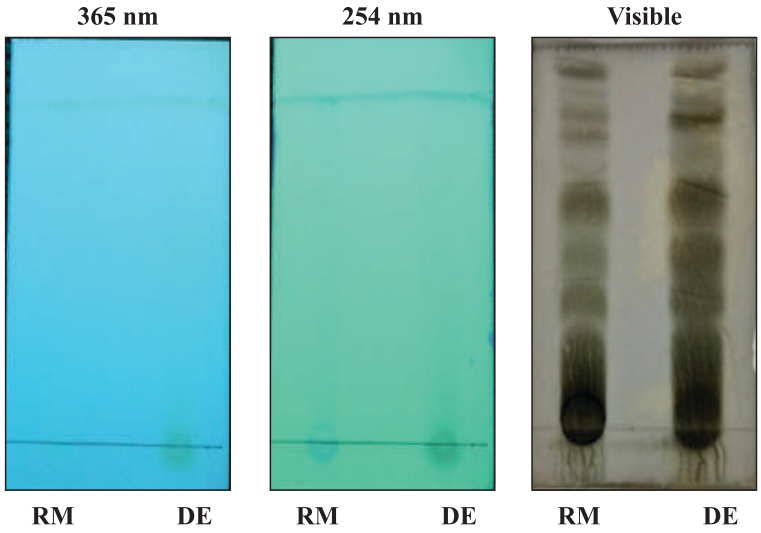
Reference : Indian Pharmacopeia 2010; P 2513-2515

Asparagus racemosus

Other Names : Shatavari

Marker Compounds : Shatavarin IV

Plant Part Used : Root



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform : Methanol (6.5 : 3.5 v/v)

Spray Reagent : Vanillin Sulphuric acid

Inference : Grey spot-0.34 ; Grey spot-0.44 ; Grey spot-0.58 ; Grey spot-0.77 ; Grey spot-0.93

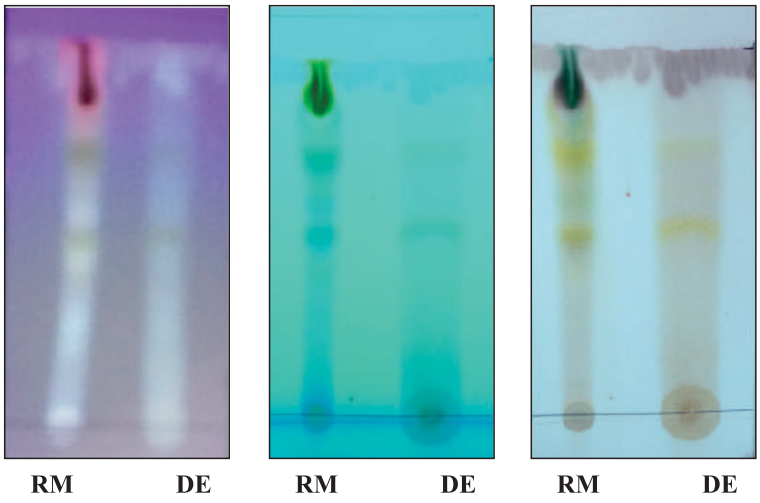
Reference : Indian Pharmacopeia 2010; P 2521-2522

Azadirachta indica

Other Names : Neem

Marker Compounds : Bitter, Rutin, Azadirachtin, Nimbedin

Plant Part Used : Leaf, Bark, Seed



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Ethyl acetate: n-butanol: Formic acid: water (25:15:5:5 v/ v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Grey spot R_f-0.23; Yellow spot R_f-0.50; Yellow spot R_f-0.63

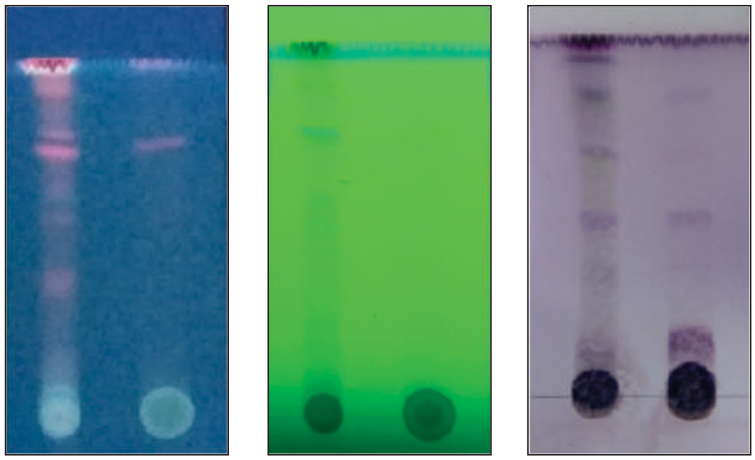
Reference : Indian Pharmacopeia 2010; P 2524-2525

Bacopa monnieri

Other Names : Brahmi, Indian pennywort, Waterhyssop

Marker Compounds : Bacoside A, BacopasideII, Bacopasaponin-C, Jujubogenin (Isomer of Bacopasaponin-C)

Plant Part Used : Whole Plant



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 2g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 50 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform : methanol (7:3 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Brown spot R_f-0.19; Purple spot R_f-0.52; Green spot R_f-0.70; Purple spot R_f-0.86; Green spot R_f-0.96

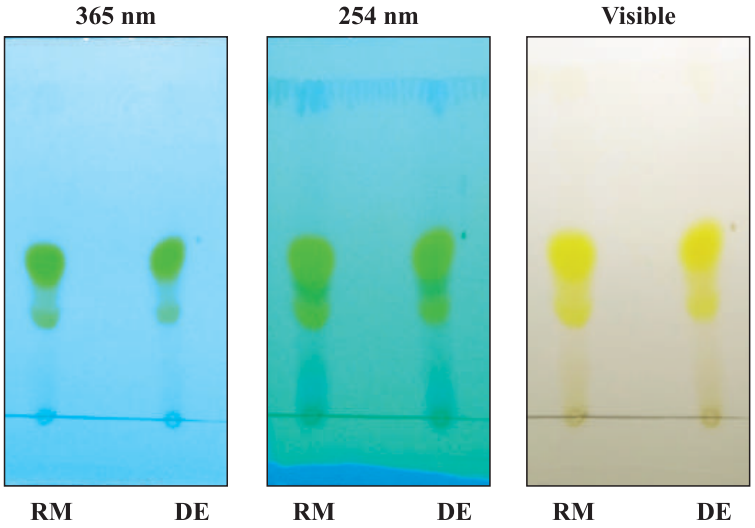
Reference : Indian Pharmacopeia 2010; P 2489-2490

Berberis arisata

Other Names : Daruharidra

Marker Compounds : Berberine

Plant Part Used : Roots



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 2g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 50 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Ethylacetate : formic acid : Glacial acetic acid : water (8:1:1:2 v/v)

Spray Reagent : Examine under 254 nm and 365 nm and also in day light.

Inference : Yellow-0.3, Yellow-0.48

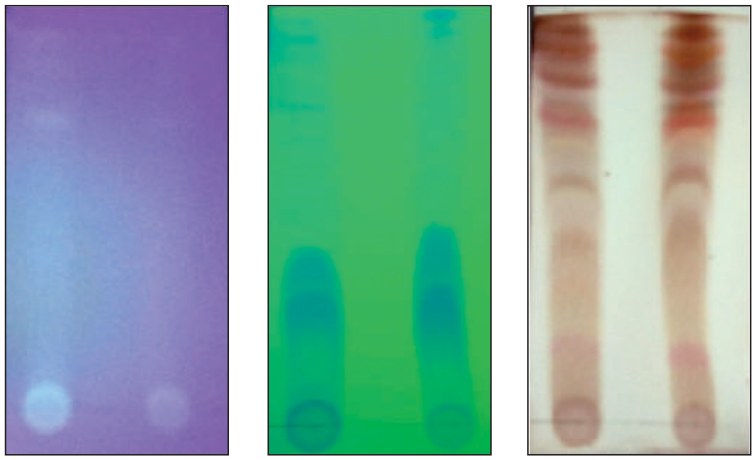
Reference : Indian Pharmacopeia 2010; P 2495-2496

Boswellia serrata

Other Names : Boswellia, Shallaki

Marker Compound : 11-Keto-ß-Boswellic Acid and Acetyl-11-keto-ß-Boswellic Acid

Plant Part Used : Gum Resin



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Hexane : Ethyl acetate (7:3 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Pink spot R_f- 0.20; Purple spot R_f- 0.47; Pink spot R_f- 0.61; Pink spot R_f- 0.76; Pink spot R_f- 0.84; Pink spot R_f- 0.93; Pink spot R_f- 0.96

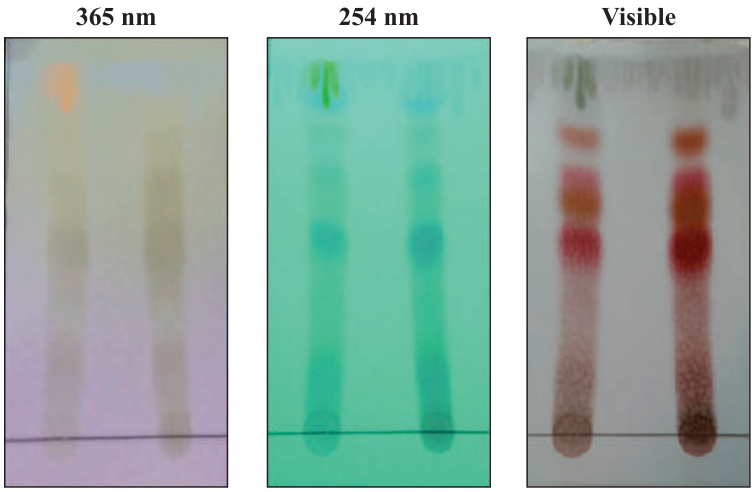
Reference : Indian Pharmacopeia 2010; P 2515-2516

Camellia sinensis

Other Names : Tea

Marker Compounds : EGCG, Caffein, Polyphenol

Plant Part Used : Leaf



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform : Acetone : Formic acid (5 : 4 : 1 v/v/v)

Spray Reagent : 1% vanillin in sulphuric acid

Inference : Red spot R_f- 0.5; Yellow R_f - 0.60; Red spot R_f - 0.68; yellow spot R_f - 0.81

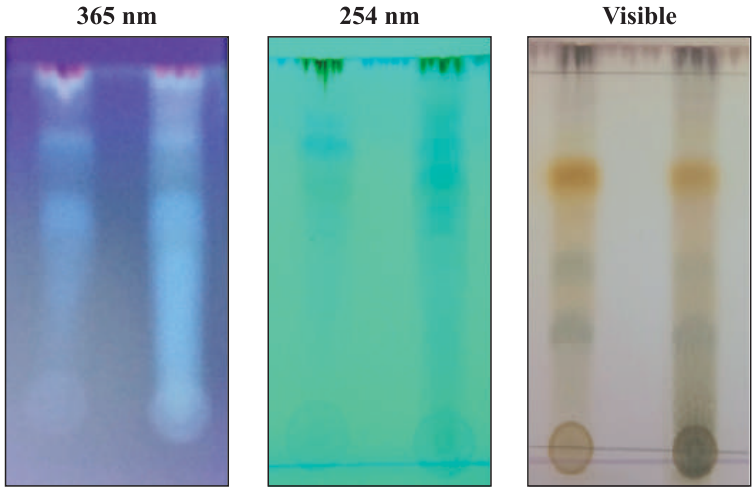
Reference : Karan Vasisht, Pritam Dev Sharma, Maninder Karan, Dev Dutt Rakesh *et all*, Study to Promote the Industrial Exploitation of Green Tea Polyphenols in India, United Nations Industrial Development Organization and the International Centre for Science and High Technology, 2003; P 1-96.

Centella asiatica

Other Names : Brahmi, Gotu kola, Mandukparni

Marker Compounds : Brahminoside, Asiaticoside, Madecassoside, Asiatic Acid

Plant Part Used : Leaf, Whole Plant



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform :Glacial acetic acid: Methanol : Water (6:3.2:1.2:0.8v/v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Green spot R_f-0.33; Green spot R_f-0.50; Brown spot R_f-0.74

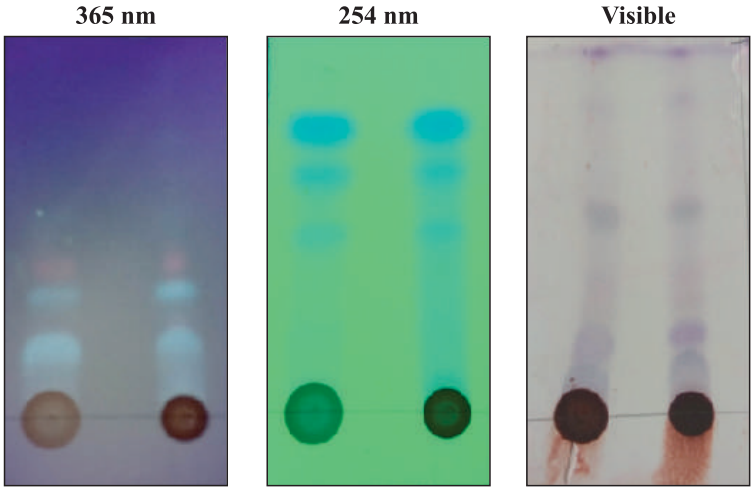
Reference : Indian Pharmacopeia 2010; P 2520-2521

Cinnamomum cassia

Other Names : Dalchini, Cinnamon

Marker Compounds : Cinnamaldehyde, Rutin, Polyphenol

Plant Part Used : Bark



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene: Ethyl acetate (19:1v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Blue spot R_f-0.15; Purple spot R_f-0.27; Purple spot R_f-0.27; Purple spot R_f-0.63; Purple spot R_f-0.90

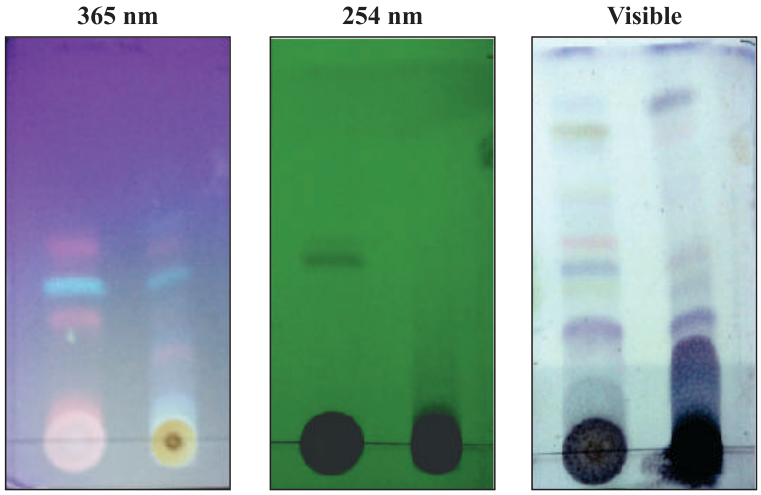
Reference : United State Pharmacopeial convention, Herbal medicine compendium, 2014

Cissus quadrangularis

Other Names : Hadjod, Asthisamharaka

Marker Compounds : Ketosterones, Calcium Oxalate, Carotene, Ascorbic Acid, Quercetin

Plant Part Used : Stem



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene: Ethyl acetate (9:1 v/v)

Spray Reagent : Vanillin sulphuric acid

Inference : Purple spot R_f-0.28; Purple spot R_f-0.36; Pink spot R_f-0.45; Purple spot R_f-0.55; Yellow spot R_f-0.78; Purple spot R_f-0.82

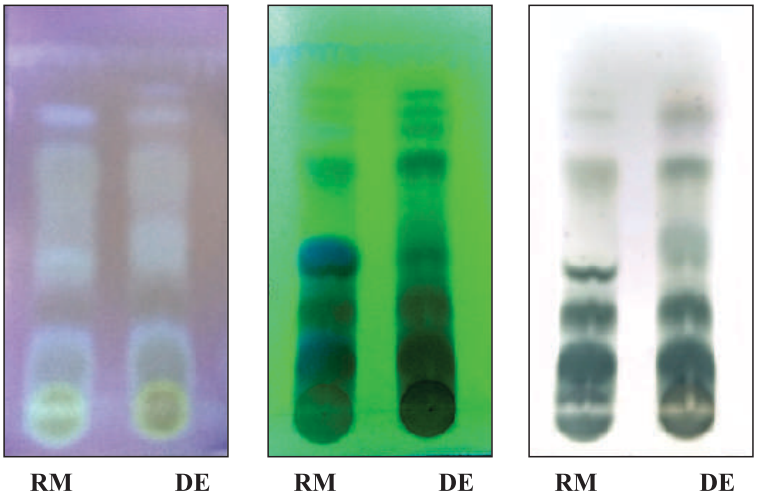
Reference : The Ayurvedic Pharmacopoeia of India, 1(3), P 21-22

Coffea robusta

Other Names : Coffee

Marker Compounds : Chlorogenic Acid, Caffeine

Plant Part Used : Bean



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene: Ethyl acetate: Formic acid: water (1.5:9.0:0.5:0.5 v/v/v/v)

Spray Reagent : 0.5% w/v ferric chloride sol in water

Inference : Green spot R_f-0.12; Green spot R_f- 0.26; Green spot R_f- 0.70; Green spot R_f- 0.84

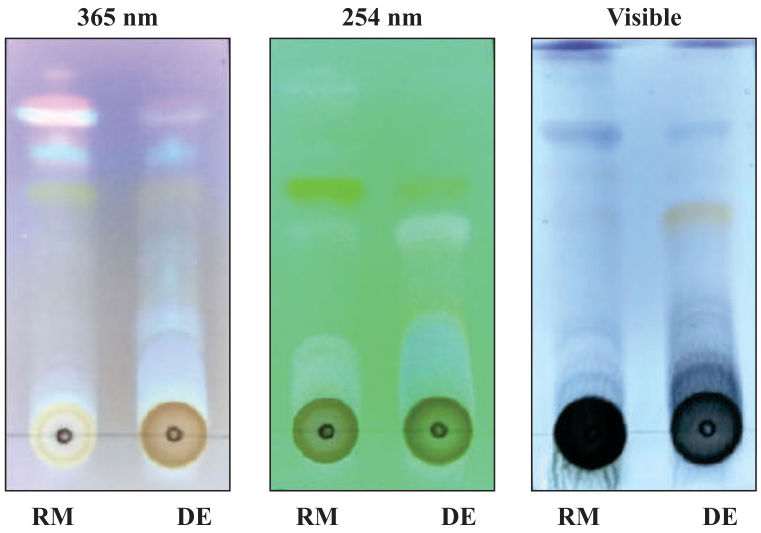
Reference : Aveen Nozad Adham, Simultaneous Estimation of Caffeic acid and Chlorogenic acid content in Ammi majus seed by TLC and HPLC, 2015, 7(6); 263-267.

Coleus forskohlii

Other Names : Coleus forskohlii, Patharchur

Marker Compounds : Forskolin, Rosmarinic acid, Flavonoid glucuronides, Diterpenoids

Plant Part Used : Root



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Ethyl acetate : Hexane (4:6 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Yellow R_f- 0.56; Red spot R_f- 0.68; Green spot R_f- 0.77

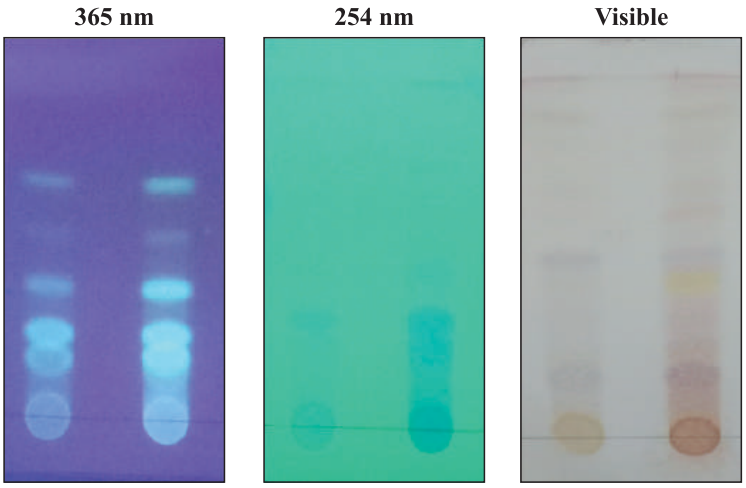
Reference : Indian Pharmacopeia 2010; P 2493-2494

Commiphora mukul

Other Names : Guggul, Indian Bdellium Tree, Mukul Myrrh Tree

Marker Compounds : Guggulsterone E&Z, Guggulipids

Plant Part Used : Gum Resin



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol.

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Petroleum ether : Ethyl acetate (9:3 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Blue R_f-0.23; Pink R_f-0.53; Grey R_f-0.92

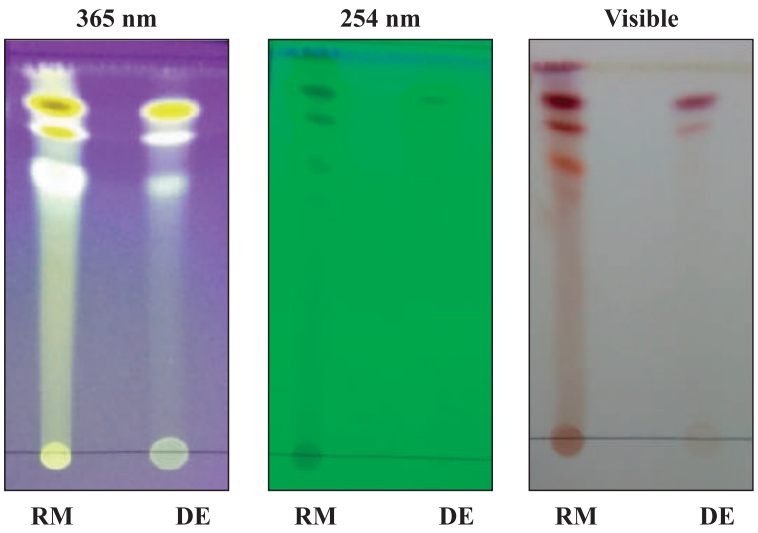
Reference : Indian Pharmacopeia 2010; P 2505-2507

Curcuma longa

Other Names : Haldi, Haridra, Turmeric

Marker Compounds : Curcumin, Bisdemethoxy Curcumin, Demethoxy Curcumin

Plant Part Used : Rhizomes



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

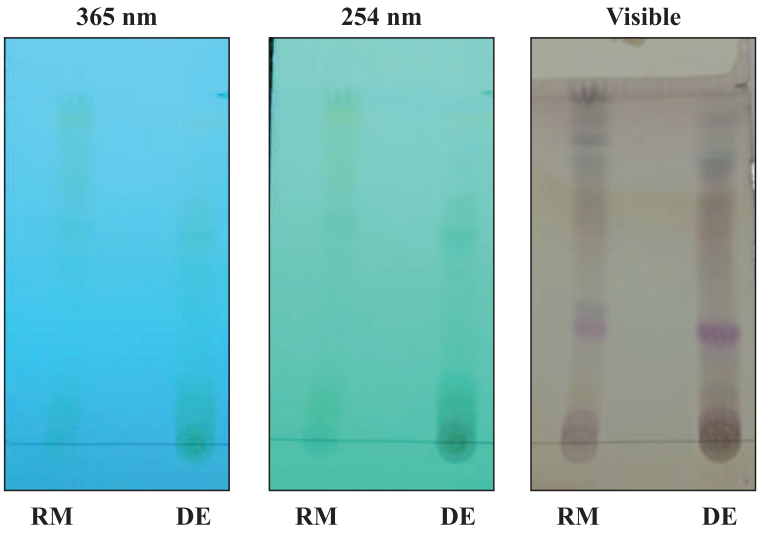
Sample Preparation	Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol
Plate Type	Silica Gel 60 F_{254}
Mobile Phase	Chloroform : Methanol : Glacial acetic acid : Formic Acid (10:1:0.5:0.5 v/v/v/v)
Spray Reagent	Anisaldehyde sulphuric acid
Inference	Yellow spot R_f-0.66; Yellow spot R_f-0.79; Yellow spot R_f- 0.86
Reference	Indian Pharmacopeia 2010; P 2507-2508

Eclipta alba

Other Names : Bhringraj

Marker Compounds : Wadelolactones

Plant Part Used : Whole Plant



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene : Acetone : Formic acid (9:6:1 v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Purple-0.04 ; Purple-0.30 ; Light Pink-0.58 ; Green-0.80 ; Grey-0.90

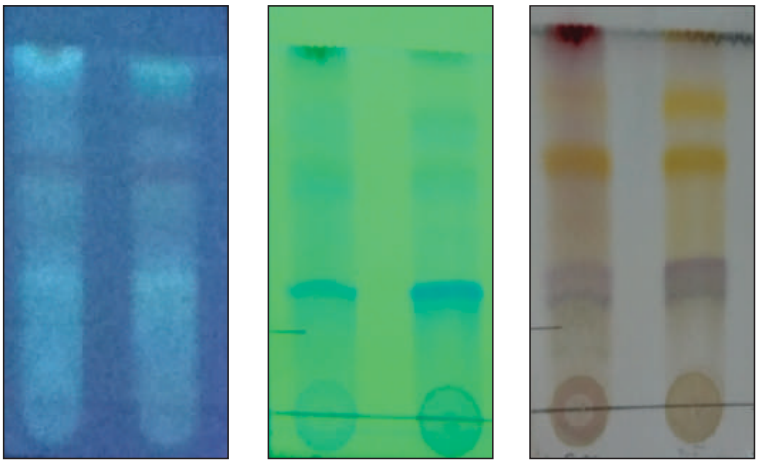
Reference : Indian Pharmacopeia 2010; P 2486-2487

Glycyrrhiza glabra

Other Names : Liquorice, Mulethi, Yashtimadhu

Marker Compounds : Glyzyrrhizin, Glabradin

Plant Part Used : Root



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol.

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : n-butanol: Acetic acid: water (7:1:2 v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Violet R_f-0.36; Yellow R_f-0.69; Yellow R_f-0.84

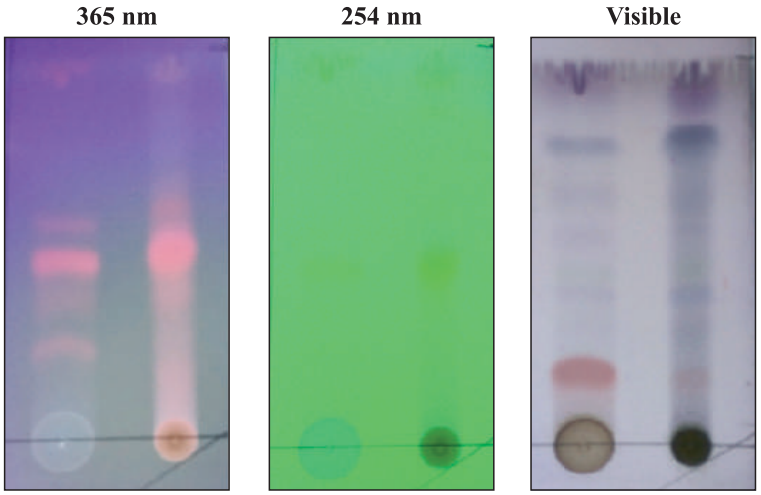
Reference : Indian Pharmacopeia 2010; P 2551-2553

Gymnema sylvestre

Other Names : Meshasringi, Gudmar

Marker Compounds : Gymnemic acid, Gymnemagenin, Gymnemasaponin

Plant Part Used : Leaf



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene: Ethyl acetate: Methanol (1:1:4 v/ v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Red spot R_f-0.15; Blue spot R_f-0.38; Blue spot R_f-0.53; Purple spot R_f-0.64; Green spot R_f-0.76

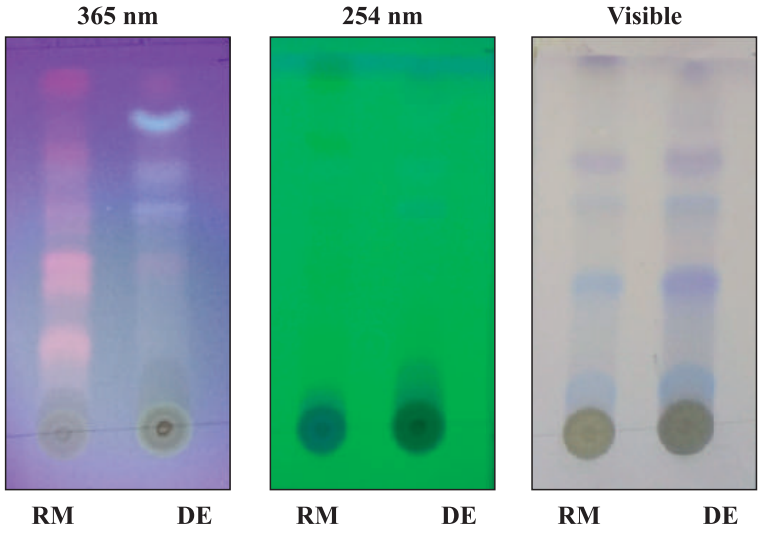
Reference : The Ayurvedic Pharmacopoeia of India, 5(1), P 128-129

Lagerstroemia speciosa

Other Names : Banaba, Jerul

Marker Compounds : Corosolic acid, Lagerstroemin

Plant Part Used : Leaf



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene, Ethyl acetate, Glacial acetic acid (55:45:0.5 v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Blue spot R_f-0.28; Purple spot R_f-0.38; Blue spot R_f-0.60; Violet spot R_f-0.71

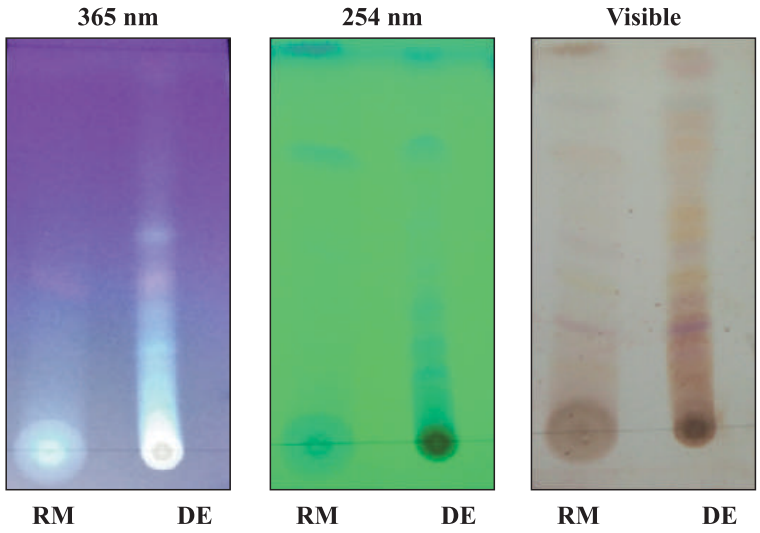
Reference : United State Pharmacopeial convention, Herbal medicine compendium, 2014

Momordica charantia

Other Names : Bitter gourd, Karela, Karavallaka

Marker Compounds : Momordicine, Charantin

Plant Part Used : Fruit



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Methanol: Benzene (2:8 v/ v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Violet spot R_f-0.18; Yellow spot R_f-0.35; Violet spot R_f-0.42; Yellow spot R_f-0.52; Blue spot R_f-0.82

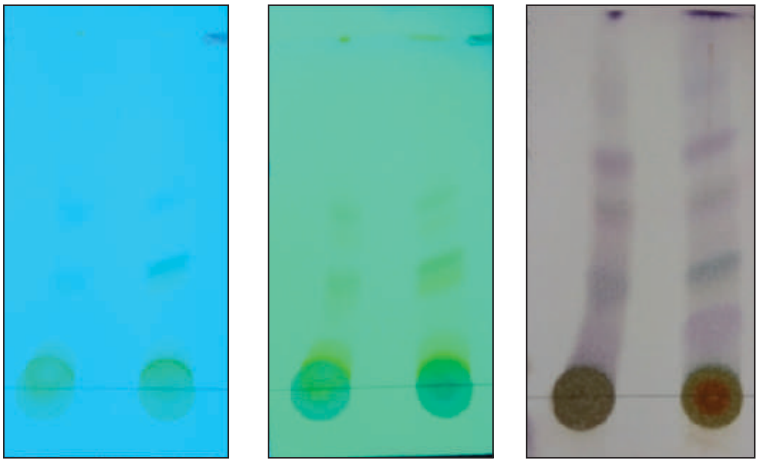
Reference : Subhash Chandra patel, Kaushal parmar, Yagnesh Bhatt, Yogesh patel, Dr. N.M. Patel, Isolation, characterization and antimicrobial, activity of charantin from Momordica charantia linn.fruit International Journal of Drug Development & Research, 2010, 2(3): P 629-634.

Moringa oleifera

Other Names : Sehjan, Mungna

Marker Compounds : Moringine, ß-sitosterol, Apigenin, Rhamnetin

Plant Part Used : Leaf, Pod



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene : Ethylacetate (9: 1v/v)

Spray Reagent : Anisaldehyde-Sulphuric acid

Inference : Purple spot R_f-0.16; Grey spot R_f-0.32; Grey spot R_f-0.37; Purple spot R_f-0.62; Purple spot R_f-0.77

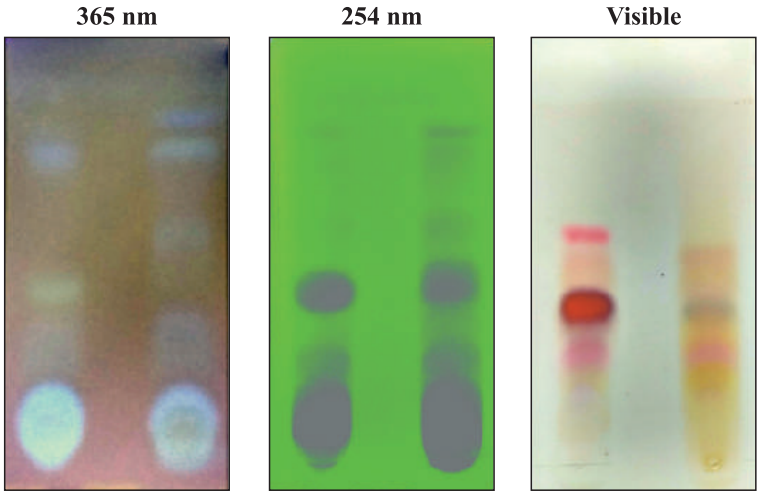
Reference : The Ayurvedic Pharmacopoeia of India, 2(1): P 163-164

Mucuna pruriens

Other Names : Kawach, Velvet bean

Marker Compounds : Levodopa

Plant Part Used : Seed



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : n-Butanol : Acetic acid: Water (4:1:5 v/v/v)

Spray Reagent : 0.2 % (w/v) Ninhdrin in ethanol

Inference : Pink spot R_f-0.28; Red spot R_f-0.41; Red spot R_f-0.58

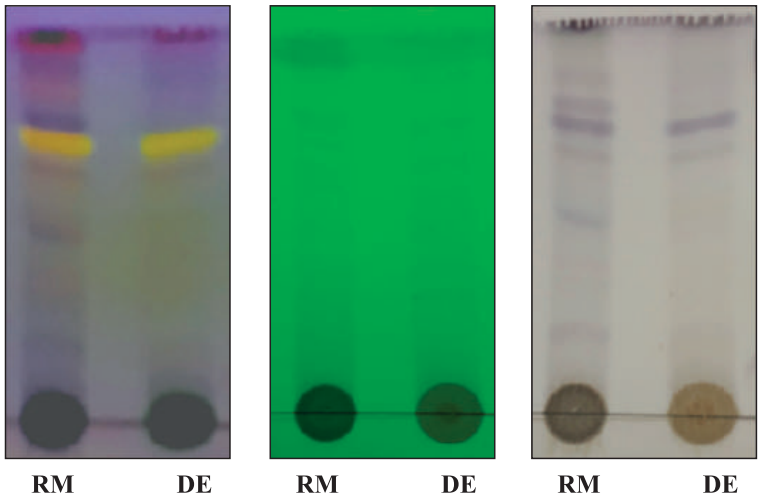
Reference : The Ayurvedic Pharmacopoeia of India, 3(1): P 23-24

Ocimum sanctum

Other Names : Tulsi, Holy Basil

Marker Compounds : Ursolic acid, Oleanolic acid

Plant Part Used : Leaves



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol.

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform: Methanol (9.5:0.5v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Purple R_f-0.29; Green R_f-0.74; Purple R_f-0.76; Pink R_f-0.83

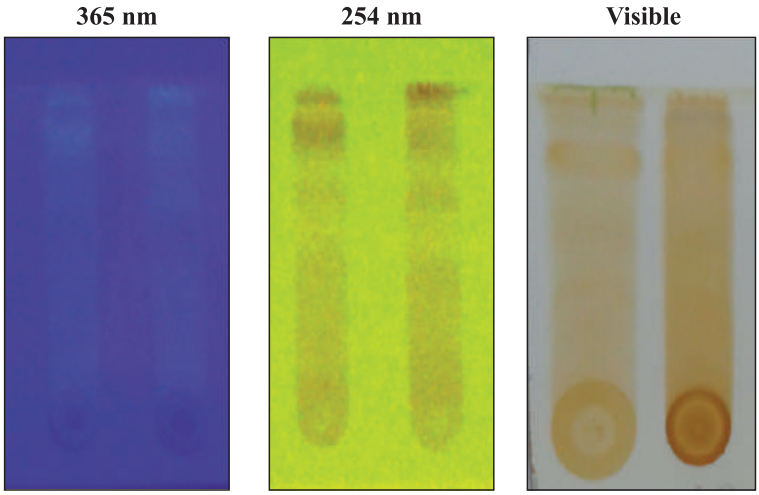
Reference : Indian Pharmacopeia 2010; P 2548-2549

Olea europaea

Other Names : Olive, Jaitun

Marker Compounds : Oleanolic acid, Rosmarinic acid, Oleuropein

Plant Part Used : Leaves



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform: Methanol : Water : Acetic acid (30:15:4:3 v/v/v/v)

Spray Reagent : Ferric Chloride solution / Vanilline sulphuric acid/Iodine vapour

Inference : Orange spot R_f-0.58; Orange spot R_f-0.66; Orange spot R_f-0.75

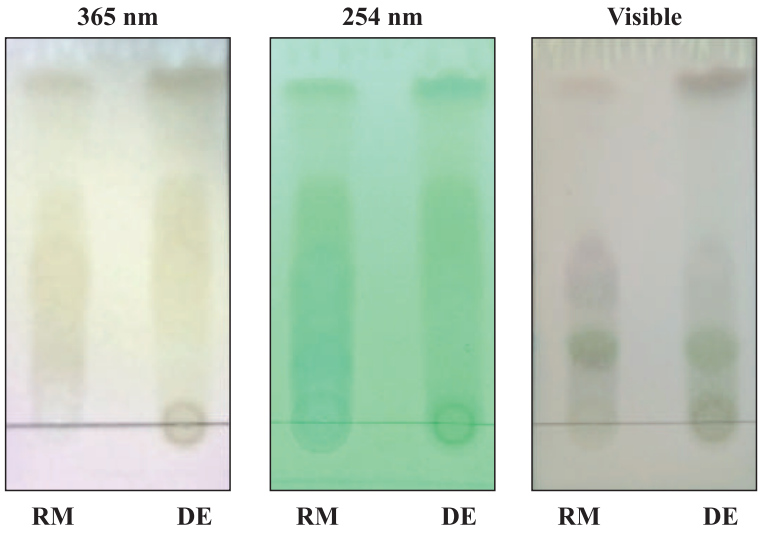
Reference : The ethanobotany and pharmacognosy of olea europea H.S. Long, P.M. Tilney, BE Vanwyk.

Phyllanthus emblica

Other Names : Indian Gooseberry, Amla, Amalika

Marker Compounds : Ascorbic Acid, Phyllanemblinin, Phyllanemblin, Gallic Acid

Plant Part Used : Fruit



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene: Ethyl acetate: Glacial acetic acid (4.5:2.0:0.5 v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Green spot R_f-0.22; Purple spot R_f-0.38; Purple spot R_f-0.91

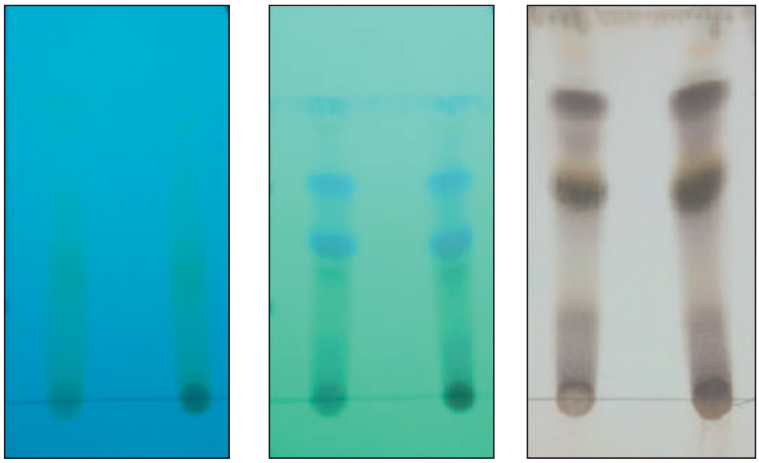
Reference : Indian Pharmacopeia 2010; P 2470-2471

Picrorhiza kurroa

Other Names : Kutki

Marker Compounds : Kutkin, Bitter

Plant Part Used : Rhizome



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Ethyl acetate: Methanol: Glacial acetic acid (7.5:2.2:0.1 v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Grey spot R_f- 0.22; Green spot R_f-0.52; Grey spot R_f- 0.64; Green spot R_f-0.78; Orange R_f-0.98.

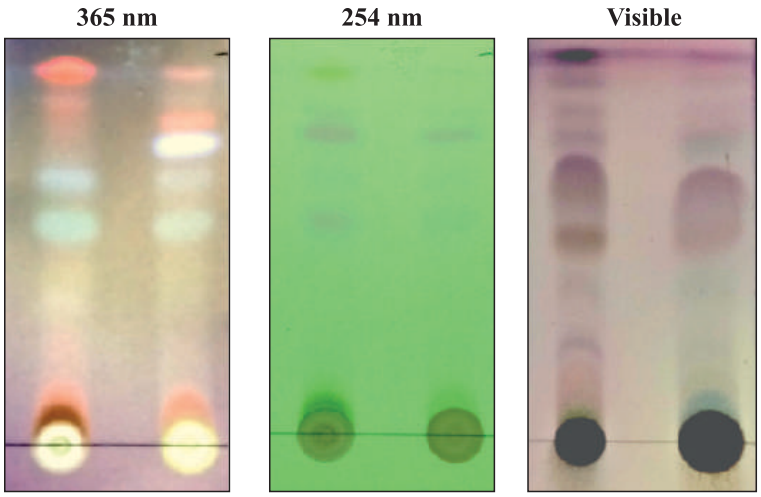
Reference : Indian Pharmacopeia 2010; P 2516-2517

Piper longum

Other Names : Pippali, Small paper, Catkins

Marker Compounds : Piperine

Plant Part Used : Fruit



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Benzene: Ethyl acetate: Diethyl ether (6:3:1 v/ v/v)

Spray Reagent : Vanillin sulphuric acid

Inference : Purple spot R_f-0.23; Purple spot R_f-0.41; Brown spot R_f-0.54; Purple spot R_f-0.70; Purple spot R_f-0.81; Purple spot R_f-0.87

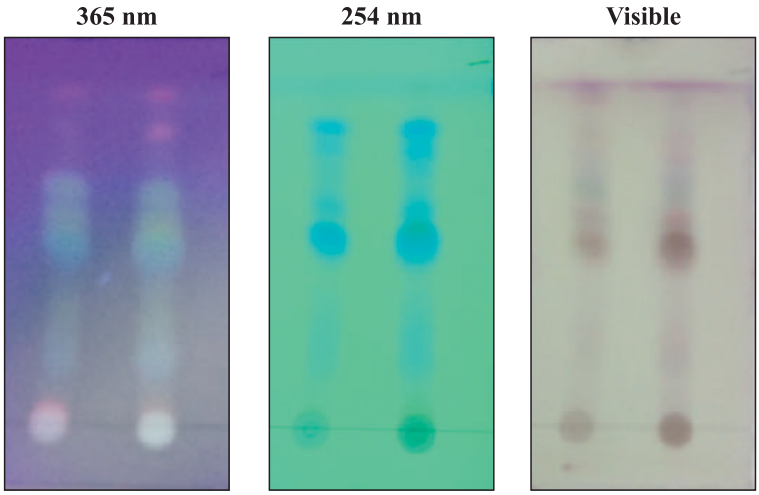
Reference : Indian Pharmacopeia 2010; P 2531-2532

Piper nigrum

Other Names : Black pepper, Maricha

Marker Compounds : Piperine, Piperdine

Plant Part Used : Fruits



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Benzene: Ethyl acetate: Diethyl ether (6:3:1 v/ v/v)

Spray Reagent : Vanillin sulphuric acid

Inference : Brown spot R_f-0.20; Brown spot R_f-0.50; Brown spot R_f-0.55; Green spot R_f-0.65; Purple spot R_f-0.68

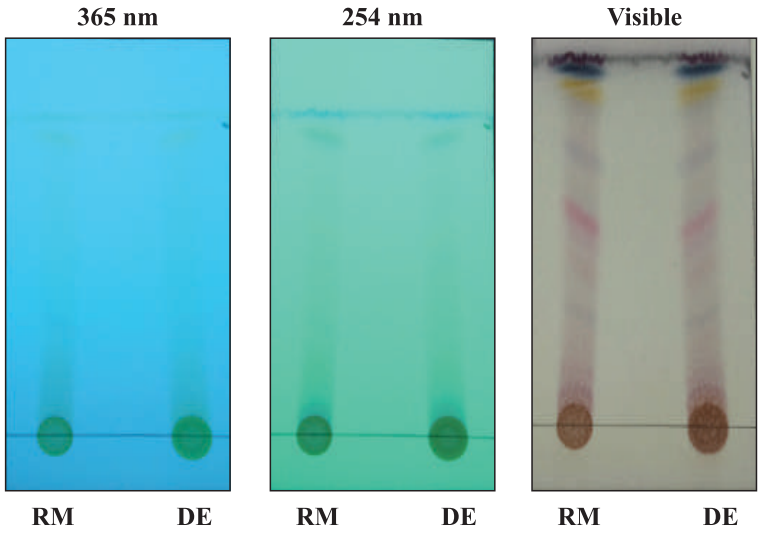
Reference : Indian Pharmacopeia 2010; P 2522-2523

Petrocarpus marsupium

Other Names : Vijaysar

Marker Compounds : Pterostilbene

Plant Part Used : Interior wood



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform : Methanol (9:1 v/v)

Spray Reagent : Vanillin sulphuric acid

Inference : Blue spot R_f-0.28; Blue spot R_f-0.42; Pink spot R_f-0.51; Purple spot R_f-0.71 ; Purple spot R_f-0.83 ; Yellow spot R_f-0.91 ; Royal Blue spot R_f-0.96 $_f$ 96

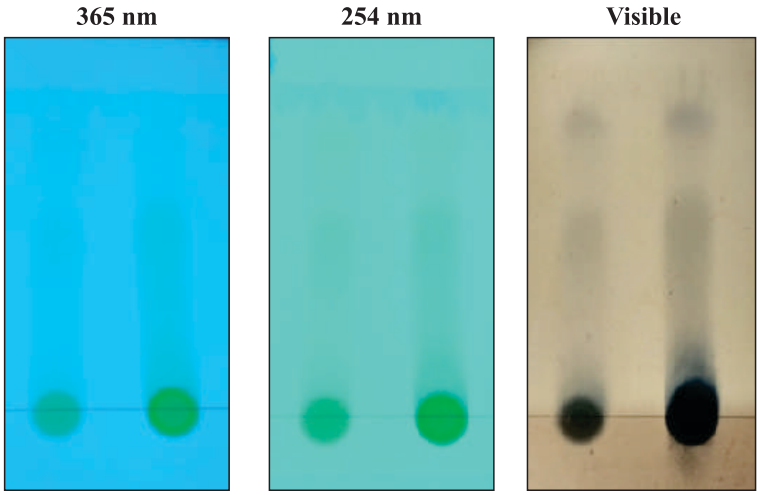
Reference : United State Pharmacopeial convention, Herbal medicine compendium, 2014

Punica granatum

Other Names : Anar, Dadima, Pomegranate

Marker Compounds : Ellagic acid, Punicalgin

Plant Part Used : Fruit rind



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform: Ethylacetate :Formic acid (5:4:1 v/v/v)

Spray Reagent : 2% Ferric chloride reagent

Inference : Black spot R_f-0.59 ; Black spot R_f-0.82

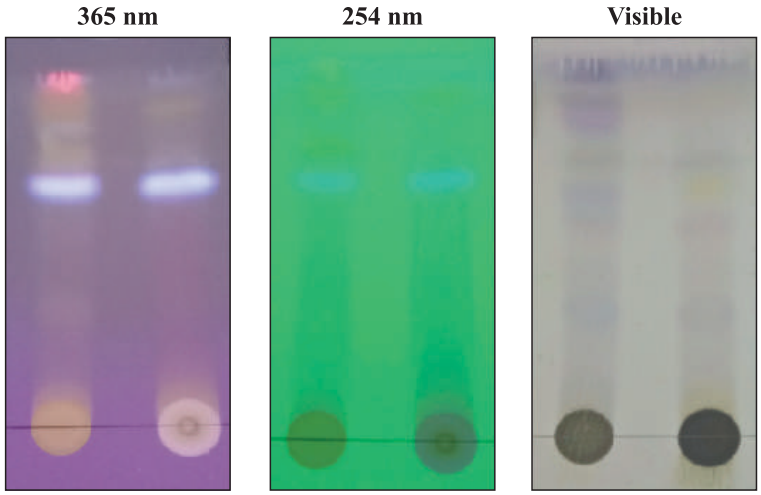
Reference : The Ayurvedic Pharmacopoeia of India, 4(1): P 22-23

Rubia cordifolia

Other Names : Manjistha, Indian Madder

Marker Compounds : Rubiadin, Munjistin

Plant Part Used : Stem



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene: Ethyl acetate: Glacial acetic acid (7:25:0.5 v/ v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Blue spot R_f-0.28; Pink spot R_f-0.52; Yellow spot R_f-0.57; Blue spot R_f-0.68

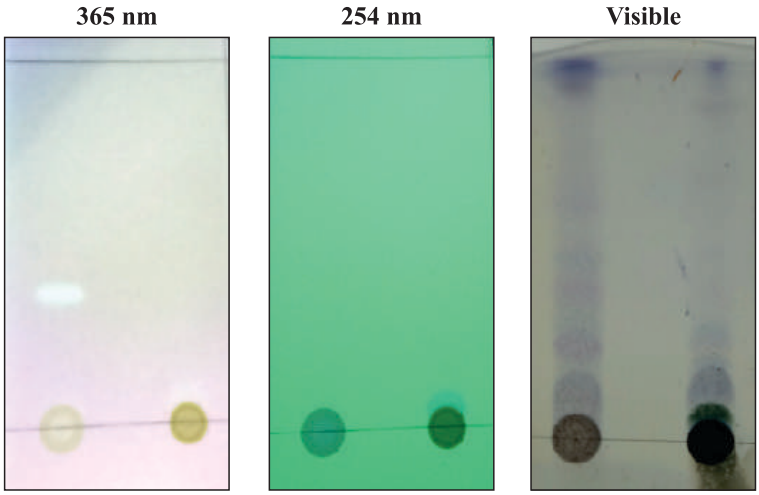
Reference : Indian Pharmacopeia 2010; P 2521-2522

Syzygium cumini

Other Names : Jamun, Jambol

Marker Compounds : Jamboline, Ellagic acid, Gallic acid

Plant Part Used : Seed / Bark



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene: Ethyl acetate (9:1 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Purple spot R_f -0.13; Red spot R_f -0.22; Red spot R_f - 0.40; Purple spot R_f -0.43; Purple spot R_f -0.93

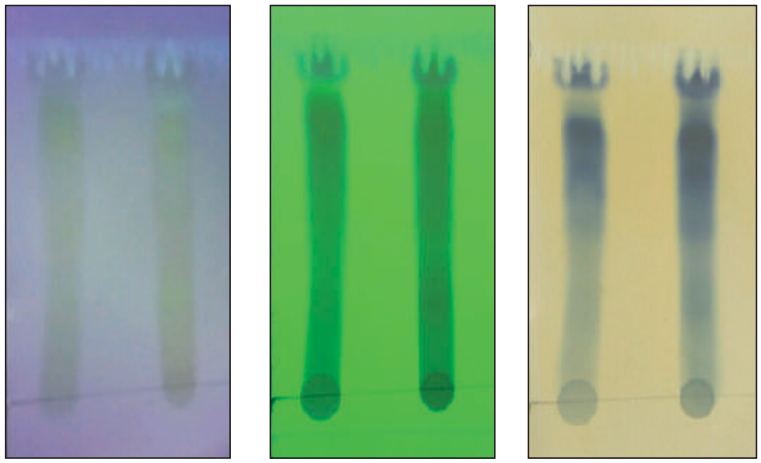
Reference : The Ayurvedic Pharmacopoeia of India, 2(1): P 57-58

Terminalia chebula

Other Names : Harad, Myrobalan, Chebulic

Marker Compounds : Chebuliagic acid, Ellagic acid

Plant Part Used : Fruit



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene : Acetone: Glacial acetic acid :Formic acid (3.5:5.0:1.5:0.5 v/v/v/v)

Spray Reagent : 1% (w/v) of Ferric chloride.

Inference : Blue spot R_f-0.28; Blue spot R_f-0.65; Blue spot R_f-0.75

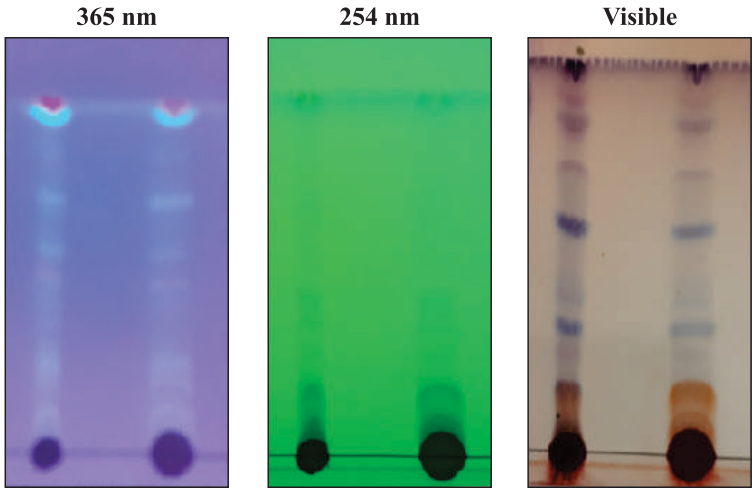
Reference : Indian Pharmacopeia 2010; P 2509-2510

Terminalia arjuna

Other Names : Arjuna

Marker Compounds : Pyrogallol, Catechol, Arjunosides, Arjunic Acid

Plant Part Used : Bark



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 2g of sample with 25 ml chloroform for 15 min, cool and filter. Reflux the residue with 50 ml chloroform. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform : methanol (9.2:0.8 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Royal Blue spot R_f-0.29; Blue spot R_f-0.41; Blue spot R_f-0.56; Blue spot R_f-0.87

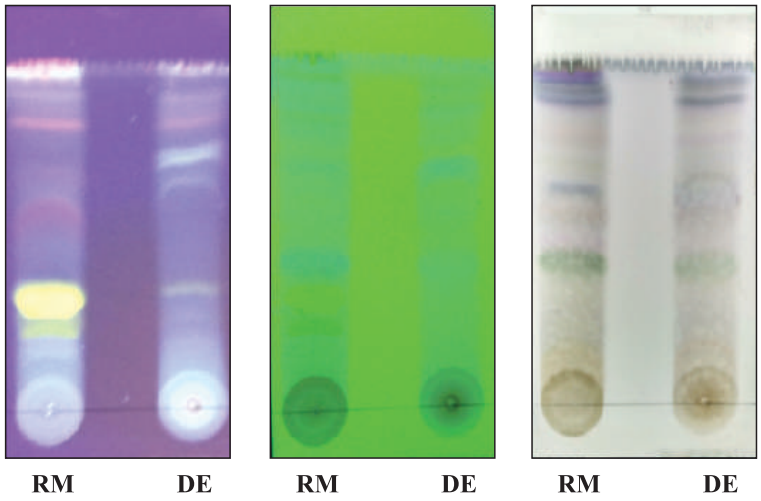
Reference : Indian Pharmacopeia 2010; P 2476-2477

Tinospora cordifolia

Other Names : Giloy, Guduchi, Heart Leaved Moonseed

Marker Compounds : Cordifolisides, Alkaloides

Plant Part Used : Stem



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol.

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform: Methanol (8.5:1.5 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Green Spot R_f- 0.40; Pink- R_f -0.46; Pink- R_f -0.54; Blue spot-0.63; Pink- R_f -0.79; Blue spot-0.88; Green Spot -R_f- 0.92; Blue spot-0.93

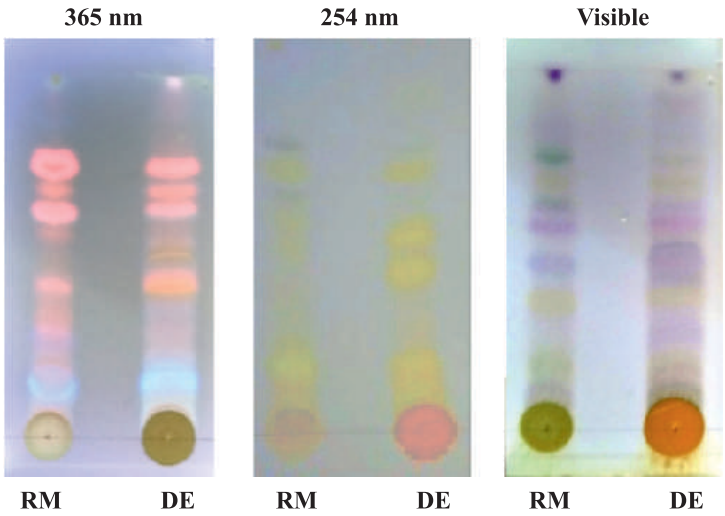
Reference : Indian Pharmacopeia 2010; P 2503-2504

Tribulus terrestris

Other Names : Gokhru, Gokhshuru, Small Calotrops, Puncture Vine

Marker Compounds : Protodioscin, Diosgenin, Steroidal Saponins

Plant Part Used : Fruit/Whole Plant



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol.

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Toluene : Ethyl acetate (8:2 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Pale green R_f-0.35; Pink R_f-0.57; Pink R_f-0.59; Pale green R_f-0.67; Green R_f-0.79

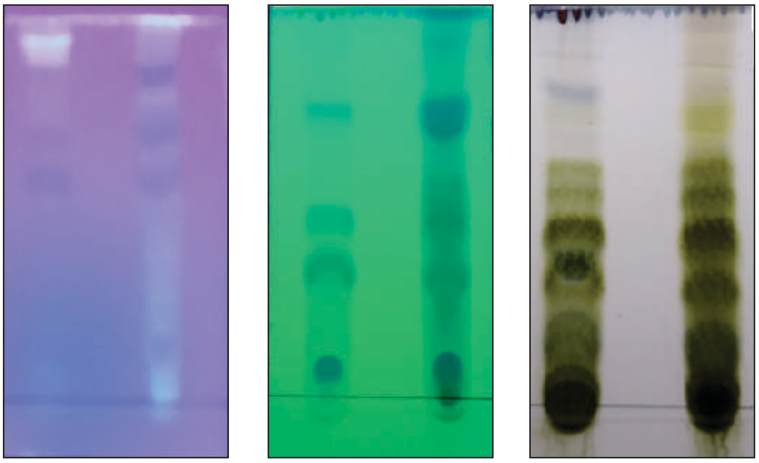
Reference : Indian Pharmacopeia 2010; P 2500-2501

Trigonella foneum graecum

Other Names : Fenugreek, Methi Dana

Marker Compounds : 4-hydroxyisoleucine, Saponins, Protodioscin

Plant Part Used : Seed



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform : methanol : water (18:8:1 v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Grey spot R_f- 0.27; Green spot R_f- 0.39; Green spot R_f- 0.51; Green spot R_f- 0.57; Green spot R_f-0.64

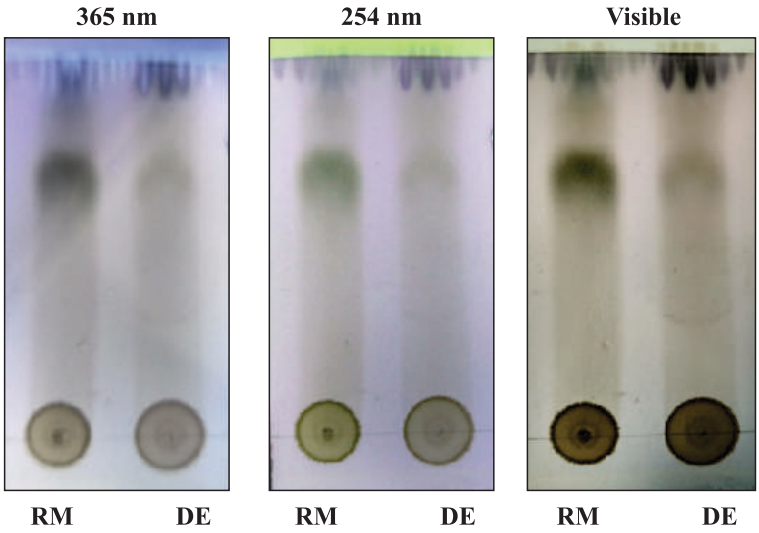
Reference : United State Pharmacopeial convention, Herbal medicine compendium, 2014

Triphala

Other Names : Combination of Harad, Baheda, Amla

Marker Compounds : Gallic Acid, Ellagic acid

Plant Part Used : Fruit



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml water for 15 min, cool and filter. Reflux the residue with 25 ml water. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in water.

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Ethanol: Glacial Acetic acid : Toluene (5.5:1:1.5 v/v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Green spot R_f-0.74

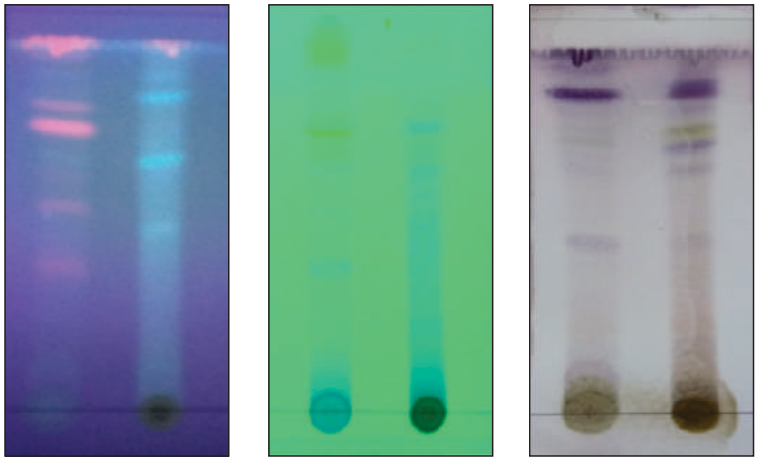
Reference : Gargi S, Mishra A, Gupta R, Fingerprint Profile of selected Ayurvedic Churnas and preparations.

Withania somnifera

Other Names : Ashwagandha, Indian Ginseng, Winter Cherry

Marker Compounds : Withanolides, Glyco withanolides, Withferin A

Plant Part Used : Root/ Whole Plant



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 2g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 50 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Chloroform : methanol (9:1 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Blue spot R_f-0.32; Purple spot R_f-0.40; Purple spot R_f-0.59; Purple spot R_f-0.83; Purple spot – 0.88

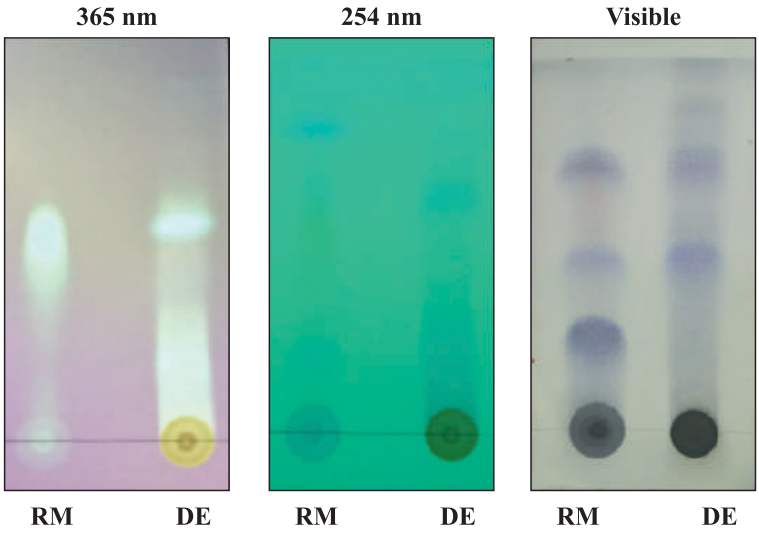
Reference : Indian Pharmacopeia 2010; P 2489-2490

Zingiber officinale

Other Names : Saunth, Sunthi, Ginger

Marker Compounds : Gingerol, Shogaol

Plant Part Used : Rhizome



Note: RM- Raw Herb ; DE- Dry Extract

TLC Method Adopted

Sample Preparation : Reflux 1g of sample with 25 ml methanol for 15 min, cool and filter. Reflux the residue with 25 ml methanol. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in methanol

Plate Type : Silica Gel 60 F_{254}

Mobile Phase : Hexane: Diethyl ether (3:7 v/v)

Spray Reagent : Anisaldehyde sulphuric acid

Inference : Purple spot R_f-0.46; Purple spot R_f-0.69

Reference : Indian Pharmacopeia 2010; P 2544-2545

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This **Thin Layer Chromatography Handbook of Indian Botanicals** is an effort to share our knowledge on validated identification techniques for herbals with our industry colleagues. It is our hope that adoption of these practices will be a step forward in understanding herbals better and consequently making better, efficacious products.

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Old Bikaner| Woollen Press Buildings| Rani Bazar| Bikaner - 334001 - India.
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