A High Performance Thin Layer Chromatographic Method Using a Design of Experiment Approach For Estimation of Phytochemical in Extracts of Selected Medicinal Plants.

A.S.Sawalkar and R.M.Kadam

Research student of Department of Botany, Shivaji College Udgir,
Head of the Department of Botany, Mahatma Gandhi College Ahmedpur,
Department of Botany Shivaji College Udgir
Swami Ramanand Teerth Marathwada University Nanded, Maharashtra, India.

Abstract: Medicinal plants are very useful for farmers because they have bioactive compounds which show antifungal and antibacterial activities against plant pathogens. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The present study involves fifteen different medicinal plants locally available in Osmanabad and Beed district of Maharashtra.

The aim of the present work was to develop a high-performance thin-layer chromatography (HPTLC) densitometric analysis technique using the different solvent systems for selected fifteen medicinal plants, which has been developed and validated for simultaneous quantification of six marker compounds (quercetin, rutin, gallic acid, ascorbic acid ,curcumin and beta sitosterol) in plant extracts. The analysis was performed on HPTLC precoated silica gel 60F254 plates with as the mobile phase (distance of 6.5 cm). Densitometric detections of marker compounds were performed at 254 and 366 nm respectively. The results of HPTLC analysis of these medicinal showed that Curcumin (Rf value 0.75) present in *Curcuma amada,* Beta sitosterol (Rf value 0.64) estimated in *Bauhinia variegata, Tinospora cordifolia, Eclipta alba, Moringa oleifera, Cassia tora, Citrus limon.* Ascorbic acid (Rf value 0.23) is exactly match with *Aegle marmelos.* Quercetin (Rf value 0.78) estimated in *Withania somenifera, Aloe vera, Psoralea cordifolia, Cassia agustifolia.* Gallic acid (Rf value 0.51) in *Azadirachta indica, Phyllanthus emblica* and *Punica granatum*. Rutin absent in *Phyllanthus emblica* and *Punica granatum*. The HPTLC technique is very important commercially and has great interest in pharmaceutical companies for the production of new drugs for curing various diseases. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants of Osmanabad and Beed will be very useful in antifungal, antibacterial activities against plant pathogens for controlling various diseases of crop plants.

Keywords: HPTLC; qualitative; Rf value; marker compound; medicinal plants.

Introduction

The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value. Plants are naturally gifted tool and the extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value (Mirghani et al. 2012) .Widespread acceptance of natural or alternative therapies by the international community requires the modernization, standardization and quality control of herbal plants, using modern science and technology (Karunamoorthi et al. 2013). Sample preparation is the crucial first step in the analysis of herbs, because it is necessary to extract the desired chemical components from the herbal materials for further separation and characterization. Thus, the development of modern sample preparation and isolation techniques with significant advantages over conventional methods for the extraction, isolation and analysis of medicinal plants is likely to play an important role in providing high quality herbal products to consumers worldwide (Pereira et al. 2004; Wu et al. 2012).

The HPTLC is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimum sample clean-up. A further major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase, unlike HPLC, thus lowering analysis time and cost per analysis (Nile & Park 2014b). The objective of the present study was to develop a simple and fast method for simultaneous determination and quantification of quercetin, rutin, Beta sitosterol, gallic acid, ascorbic acid and Curcumin (Figure 1) in Aegle marmelos, Aloe vera, Eclipta alba, , Punica granatum, and Psoralea coryfolia, Bauhinia variegata, Tinospora cordifolia, Moringa oleifera, Azadirachta indica, Withania somenifera, Cassia tora, Curcuma amada, Cassia agustifolia, Emblica officinalis, Citrus limon, extracts using HPTLC. These are important medicinal plants in Ayurveda and they have been used against various diseases and disorders (Nile & Khobragade 2010).

Medicinal plants used for phytochemical analysis are –

1 .*Aegle marmelos:*

Family: Rutaceae

It has been used for centuries as an herbal medicine. It is commonly known as Bael, is indigenous to India and is one of the most useful medicinal plants in India. Its stem, bark, root, leaves and fruits have medicinal value. Other actions like antifungal, antibacterial, antiprotozoal, hypoglycemic, antioxidant, antiviral and cardioprotective effects have been studied using various parts of the plant. Besides its antioxidant properties, *Aegle marmelos* unripe fruit aqueous extract interacts by various other mechanisms in a complex way to elicit its therapeutic effects.

2. Aloe (*Aloe vera*)

Family: Liliaceae

It is the oldest medicinal plant and the most applied medicinal plant worldwide. It has numerous monosaccharides and polysaccharides; vitamins B1, B2, B6, and C; niacinamide and choline, several inorganic ingredients, enzymes (acid and alkaline phosphatase, amylase, lactate dehydrogenase, lipase) and organic compounds (aloin, barbaloin, and emodin). *Aloe vera* helps to soothe skin injuries affected by burning, skin irritations, cuts and insect bites, and its bactericidal properties relieve itching and skin swellings, wrinkles and actively repair the damaged skin cells that cause the visible signs of aging. Aloe is a powerful detoxifier, antiseptic and tonic for the nervous system, immune-boosting and anti-viral properties.

3 .*Eclipta alba* (L.)

Family: Asteraceae

It is commonly known as bhringraj as well as false daisy. It is a weed which grows in tropical and subtropical regions all over the world. It is widely dispersed throughout India, Brazil, Thailand, and China. The herb *Eclipta alba* contains many bioactive components such as coumestans i.e. wedelolactone and demethylwedelolactone, triterpenes, flavonoids, steroids, polypeptides, polyacetylenes and thiophene-derivatives. The plant is commonly used in hair oil all over India for healthy black and long hair, catarrhal jaundice and for skin diseases. *Eclipta alba* constituents of wedelolactone were investigated in the myotoxicity of snake venoms. The herb has been known for its curative properties and utilized as antimytotoxic, analgesic, antibacterial, antihepatotoxic t6, antihemorrhagic, antihyperglycemic, antioxidant, immunomodulatory properties and it is considered as a good rejuvenator too.

4. *Punica granatum*

Family: Lythraceae

Pomegranate is the common name of the ***Punica granatum*** (PG) and belongs to the family Lythraceae. It has much medical significance and has been used as medicine for centuries [16]. The recent studies have investigated that pomegranates are used for the treatment of a number of diseases e.g., diabetes, dysentery, diarrhea, cough, asthma, bleeding disorders, bronchitis, fever, AIDS, inflammation, ulcers, malaria, prostate cancer, hypertension, atherosclerosis, hyperlipidemia, male infertility, infant brain ischemia and obesity.

5. *Psoralea corylifolia*

Family: Leguminosae

Subfamily: Papilionaceae

It is an annual herb growing throughout the plains of India. The plant immense biological importance, and it has been widely exploited since ages for its magical effect against several skin diseases, such as psoriasis, leucoderma and leprosy. ***P. corylifolia*** extracts have found to possess antitumor, antihyperglycemic, antidepressant and antioxidant activities. Its water extract possesses antibacterial properties. Seeds and extract powder are used as diuretic, anthelmintic, laxative and for healing wounds. The major components psoralean and isopsoralean have antitumor, antibacterial and antiviral properties [17].

6. *Bauhinia variegata*

Family: Caesalpiniaceae

Botanical Origin (Kirtikar and Basu, 1994) ***Bauhinia variegata*** Linn. *Bauhinia variegata* Linn (Mountain Ebony) is a medium-sized, deciduous tree, found all through India, climbing to a height of 1,300 m in the Himalayas. The plant is generally utilized by the tribals all through India and mainstream in different indigenous frameworks of drugs like Ayurveda, *Bauhinia variegata* Linn. are hostile to diabetic, against ulcer, hostile to oxidant, nephroprotective, hostile to growth, hepatoprotective, calming, and immunomodulatory, hostile to microbial, hostile to bacterial.

7. *Tinospora cordifolia*

Family: Menispermaceae

Gulvel is a medicinal plant with various properties of importance for our health. It is a deciduous shrub commonly called “Guduchi” in Sanskrit. Gulvel has several beneficial properties. It may have effective properties on immunity (immunomodulatory), anti-toxin, antidiabetic, antioxidant, antiarthritic, anticancer, antimicrobial activity, and anti-inflammatory properties.

8. *Moringa oleifera*

Family: Moringaceae

Drumstick tree has numerous traditional medicinal uses in many parts of its native and the tree continues to have an important role in traditional Asian and West African medicine. In traditional Indian medicine various parts of the tree are used therapeutically, including for treatment of ascites, rheumatism, venomous bites, and as cardiac and circulatory stimulants. The roots, leaves and seeds are of particular importance in Ayurveda, and the uses of the roots, root bark, stem bark, stem exudates, leaves, flowers, and seeds treating a wide range of ailments

The flowers are also used as a tonic, diuretic and cholagogue. The leaves, rich in vitamin A and C, are considered useful in scurvy and respiratory ailments; they are also used as an emetic. The juice extracted from the leaves has strong antibacterial and antimalarial properties.

9. *Azadirachta indica*

Family: Meliaceae

Neem ingredients are applied in Ayurveda, Unani, Homeopathy, and modern medicine for the treatment of many infectious, metabolic, or cancer diseases. Different types of preparation based on plants or their constituents are very popular in many countries in disease management. In this vista, neem (*Azadirachta indica*), a member of the Meliaceae family, commonly found in India, Pakistan, Bangladesh, and Nepal, has therapeutics implication in diseases cure and formulation based on the fact that neem is also used to treat various diseases.

Azadirachta indica has complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids and such types of ingredients play role in diseases management through modulation of various genetic pathways and other activities. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves and were known to have antifungal and antibacterial activity [17].

10. *Withania somnifera*

Family: Solanaceae

It is an important medicinal plant of the Indian subcontinent. It is a woody shrub commonly known as “Indian ginseng” or “winter cherry.” It is also known as ashwagandha in Sanskrit and asgandh in Urdu (Dhuley, 1998; Ziauddin et al., 1996). It was widely used alone or in combination with other herbs to treat numerous biological problems in humans. It possesses a wide spectrum of pharmacological properties, such as antimicrobial, anti-inflammatory, antistress, antitumor, cardioprotective, and many more for use in the treatment of biological approaches. Thus, the plant and its steroidal components mitigate pathophysiological aspects of the disease; still, further studies are needed to prove the safety and efficacy of this compound in humans [18].

11. Cassia tora Linn.

Family: Leguminosae

It is a well-known plant widely distributed in India and other tropical countries. It is an annual shrub and grows in wild wasteland. Different parts of the plant (Leaves, seed, and root) are reputed for their medicinal value. It is well recognized in traditional medicine as laxative and is useful for treatment of leprosy, ringworm infection, ophthalmic, skin diseases and liver disorders. Several chemical compounds such as Anthraquinone glycosides, Naphthopyrone glycosides, Phenolic compounds, Flavonoids etc. have been isolated from this plant Properties are antibacterial, antifungal, and antioxidant, anti-inflammatory.

Antifungal Activity: The leaf extract has shown the significant antifungal activity to inhibit the growth of *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae* and *Trichophyton mentagrophyte*. It shows antifungal activity due to chrysophanol and chrysophanic acid- 9- anthrone and other anthraquinones such as emodine, physcion and rhein [19].

12. *Curcuma amada* (mango ginger)

Family: Zingiberaceae

It is a rhizomatous aromatic herb cultivated throughout India, Sri Lanka, Bangladesh and in many South-East Asian countries .The rhizomes are bitter, sweet, sour aromatic (a mixture of tastes, starting from bitter initially, turning to a sweet and then sour aromatic sensation), and cooling; used as an appetizer, carminative, digestive, stomachic, demulcent, febrifuge, alexeteric, aphrodisiac, laxative, diuretic, expectorant, anti-inflammatory and antipyretic and used in the treatment of anorexia, dyspepsia, flatulence, colic, bruises, wounds, chronic ulcers, skin diseases, pruritus, fever, constipation, hiccough, cough, bronchitis, sprains, gout, halitosis, otalgia and inflammations (Hussain *et al*., 1992; Warrier *et al*., 1994).

There is only very limited literature available on the pharmacological activity of the extract (Bhakuni *et al*., 1969; Rao *et al*., 1989). The rhizome extract of the plant exhibited a hyper-cholesteremic effect in rabbits (Pachuri and Mukherjee, 1970). The extract showed presence of an antibiotic principle with strong inhibitory activity on *Aspergillus niger* and *Trichophyton rubrum* (Gupta and Banerjee, 1972).

13. *Cassia angustifolia* (Senna)

Family Fabaceae

It is used as a laxative, and is a plant from the Fabaceae family. It includes hydroxyanthracene glycosides, also known as Senna Sennoside. In Brazilian traditional medicine, botanical species belonging to the genera *Senna* and *Cassia* are widely used as laxative, analgesic, and antifungal agents to treat ringworm and other fungal skin infections.

14. *Emblica officinalis or Phyllanthus emblica*

Family: Euphorbiaceae

Indian gooseberry or amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda and in various traditional folk systems of medicine in the Southeast Asia. The fruits are a rich source of vitamin C and are of dietary and culinary use in India.
In the traditional system of medicine, amla is used to treat a variety of ailments Scientific studies have shown that amla possesses antibacterial, antifungal, antiviral, antidiabetic, antiulcerogenic, antioxidant, antimutagenic, anti-inflammatory, immunomodulatory, antipyretic, analgesic, a antiatherogenic, gastroprotective, antianemia, anti hypercholesterolemia, wound healing, antidiarrheal, antiatherosclerotic, nephroprotective, neuroprotective and hepatoprotective properties [21].

15. *Citrus limon* (L.)
Family: Rutaceae
It is popularly known as the lemon tree is a species from the Rutaceae family, native to Asia. Lemon fruit is a rich source of nutrients, a key to a healthy diet, and provides health benefits. Lemons are an interesting source of flavonoids, vitamins, minerals, dietary fibers, essential oils, organic acids, and carotenoids. The nutritional composition, bioactive compounds, antioxidant properties, and other factors, such as antinutritional compounds and contaminants that may enter the fruit production chain, such as pesticides and heavy metals [22].
The main objective of our research work was to analyze the presence or absence of different phytochemicals in the selected fifteen medicinal plants used for antifungal activity.

MATERIALS AND METHODS

Collection of plant materials Fresh parts of fifteen medicinal plants were collected from different regions of Osmanabad and Beed districts of Maharashtra.

Sr no.	Plant Name	Common Name	Parts used
1.	***Aloe vera***	Korphad	Leaves
2.	***Aegle marmelos***	Bael	Leaves
3.	***Psoralea coryfolia***	Bawarchi	Seed
4.	***Bauhinia variegata***	Kanchanar	Bark
5.	***Tinospora cordifolia***	Gulvel	Leaves
6.	***Eclipta alba***	Bhringraj	leaves
7.	***Withania somnifera***	Ashwagandha	Leaves
8.	***Azadirachta indica***	Kadulimb	Leaves
9.	***Moringa oleifera***	Shevga	Leaves
10.	***Cassia angustifolia***	Sonamukhi	Leaves
11.	***Curcuma amada***	Ambehalad	Rhizome

12.	***Cassia tora***	Tarvat	Leaves
13.	***Punica granatum***	Dalimb	Leaves
14.	***Phyllanthus emblica***	Amla	Leaves
15.	***Citrus limon***	Limbu	Leaves

Table. I Ethnobotanical information of selected medicinal plant species for phytochemical analysis.

The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using a mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

Preparation of plant extracts

Solvent extraction Crude plant extract was prepared by the Maceration extraction method.

About 10gm of powdered plant material was placed in a stoppered container with 100ml Ethanol solvent and allowed to stand at room temperature. For a period of at least 3 days with frequent agitation until the soluble matter dissolved. The mixture was strained, the marc (the damp solid matter) was pressed, and the combined liquids were clarified by filtration. The filtrate was used for the phytochemical analysis.

l.Chemicals, Equipments and Instruments:

Equipments: Measuring cylinder, beakers, micropipette, tissue paper, Aluminium foil, eppendorf tubes, paraffin wax, TLC Plate precoated silica gel aluminium plate 60F254 (20x10), scale, Pencil, Camag 500microlite sample syringe.

Chemicals: All chemicals of analytical grade were used.

Toluene, Ethyl acetate, formic acid, Glacial Acetic acid, Methanol.

Standard compounds of sigma grade Beta sitosterol, Quercetin, Ascorbic acid, Rutin, Gallic acid and curcumin were used.

Instruments:

UV chamber, HPTLC system used for analysis was of CAMAG HPTLC system, Camag Linomat 5 sample applicator (Switzerland), On Camag TLC scanner 3.

Preparation of Standard solution:

Stock solutions of Beta sitosterol, Quercetin, Ascorbic acid, Rutin, Gallic acid and curcumin (1mg/ml) were prepared in methanol and sonicate for 5 minutes respectively .Different concentrations were applied on TLC plate, Linomat V for preparing calibration curve.

Mixture of standard stock solution: 100ul from each standard stock solution were taken in eppendorf tube.

Preparation of working standard solution:

100ul standard stocks of each standard compound diluted in 400ul methanol were applied on TLC plate.

Mixture of working standard stock solution: 50ul from each working standard solution were taken in Eppendorf tube.

Different concentrations of standard stock solution and standard mixtures were applied on TLC Plate.

Sample preparation: 1gm powered herbal samples of 15 medicinal plants were extracted with10 ml methanol for overnight in shaker. The supernatant was filtered through a 0.45um filter for TLC analysis.

Efficiency of different solvents system for separation of standard compounds by TLC.

The analytical separation of standard compounds by TLC was investigated using silica gel F 254 plates and developed with different solvent system. The different solvent system were tested for TLC including

The elution system toluene, ethyl acetate, formic acid, acetic acid, chloroform, methanol, toluene, acetone, glacial acetic acid. The method was selected according to Rf values of each standard compound and TLC plates were observed under UV chamber at 254nm and 366 nm. The photograph of each TLC plate were taken for observation of band resolution.

Standard compounds	Extract Samples	Mobile phase
B- sitosterol	4.*Bauhinia variegata* 5.*Tinospora cordifolia* 6.*Eclipta alba* 9. *Moringa oleifera* (Shevga), 12. *Cassia tora* (Tarvat) 15. *Citrus limon(* limbu)	Toluene: Ethyl Acetate: Formic Acid (7:2.5:0.5)
Curcumin	11. Curcuma amada	Chloroform: Methanol (9.5:0.5)
Ascorbic Acid, Quercetin and Gallic Acid	2. *Aegle marmelos* 7.*Withania somnifera* 8.*Azadirachta indica* 1. *Aloe vera* 3. *Psoralea coryfolia* 10. *Cassia agustifolia*	Toluene: Acetone: Formic Acid (4.5:4.5:1)
Rutin, Gallic acid and Quercetin	13. *Prunus granatum* 14. *Phyllanthus emblica*	Toluene: Acetone: Glacial Acetic Acid (4.5:4.5:1)

Optimization of the HPTLC method: It is well known that the solvent do exert a profound influence on the quality and shape of the peak; the chromatographic separation studies were carried out on the stock solution of Beta sitosterol, Quercetin, Ascorbic acid, Rutin, Gallic acid and curcumin Initially on the plate 10ul of stock solution was applied as band 8mm of width. Plates were developed using different solvent system as shown in table. Based on the results of these initial chromatograms ternary and secondary mixtures of solvents were tried to achieve optimum resolution in standard compounds.

After several trials mixture of Toluene: Ethyl Acetate: Formic Acid (7:2.5:0.5) was suitable for beta-sitosterol and extracts sample no.4, 5, 6, 9, 12.and 15. Chloroform: Methanol (9.5:0.5) for Curcumin, Curcuma amada, Toluene: Acetone: Formic Acid (4.5:4.5:1) for Ascorbic Acid, Quercetin and Gallic Acid and extracts no. 1,2,3,7,8,10. Toluene: Acetone: Glacial Acetic Acid (4.5:4.5:1) for Rutin, Gallic acid and Quercetin. And extracts sample no. 13.14. were chosen as the mobile phase for analysis then the plate was dried in oven at 60°C for 5minute. The other chromatographic conditions like chamber saturation time, run length, sample application rate and volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible Rf value, better resolution, and symmetrical peak shape for the standard stock solutions and standard mixtures.

HPTLC procedure for identification of herbal samples

1gm powder herbal samples of 15 medicinal plants were extracted with 10ml methanol for overnight in shaker. The supernatant was filtered through a 0.45um filter for TLC analysis.

The samples and standard stock mixture solution were spotted in the form of bands with a Camag 500microlite sample syringe (Hamilton, Bondouz, Switzerland) syringe on precoated silica gel aluminium plate 60F254 (20X10) with 250µm thickness;(Merck") using a Camag Linomat 5 sample applicator (Switzerland).The linear ascending development was carried out in 20cmX 10cm twin trough chamber using mobile phases. TLC plates were dried in hot air oven. TLC plates were observed under UV chamber at 254nm and 366 nm. The photograph of each TLC plate were taken for observation of band resolution. Densitometric scanning was performed on Camag TLC scanner 3 in the reflectance-absorbance mode after development for all measurement and operated by Camag ***WINCATS*** software. The source of radiation utilized was tungsten lamp and D2 lamp emitting a continuous UV spectrum 366nm and 254nm respectively.

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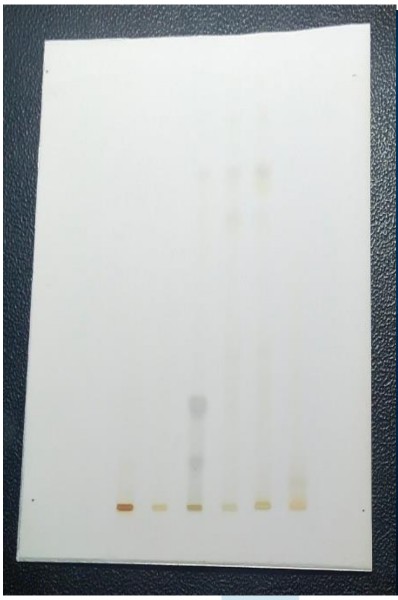
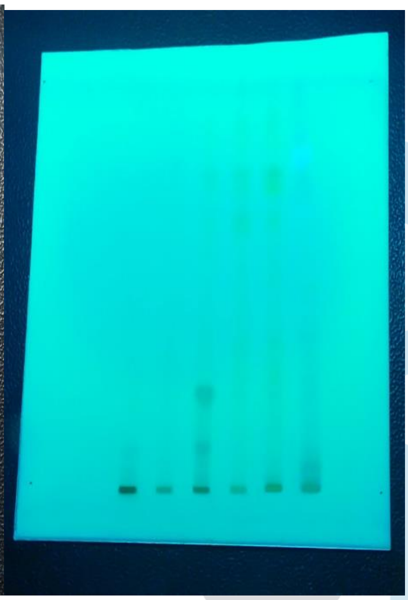
Results and Observations:

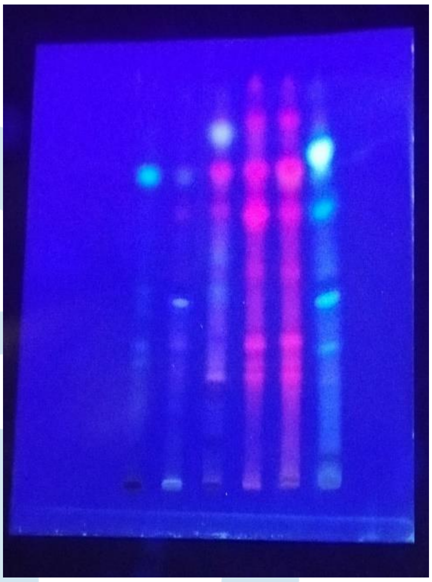
Observation 1.



Under visible light



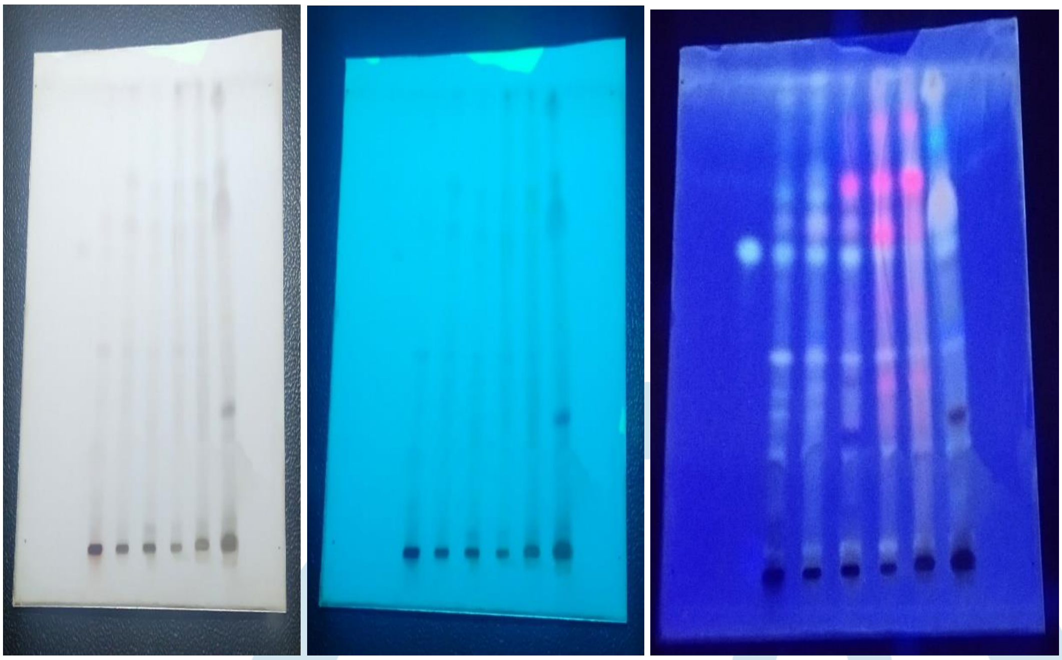
short U.V at 254 nm



Under U.V. at 366nm

- Track 1: Blank (Methanol; 2 µL)
- Track 2: B-Sitosterol (Concentration 4000 ng/band)
- Track 3: Extract 4. (Volume Applied 10 µL)
- Track 4: Extract 5. (Volume Applied 10 µL)
- Track 5: Extract 6.(Volume Applied 10 µL)
- Track 6: Extract 9.(Volume Applied 10 µL)
- Track 7: Extract 12. (Volume Applied 10 µL)
- Track 8: Extract 15 .(Volume Applied 10 µL)
- Track 9: Blank (Methanol; 2 µL)

After Derivatization with Vanillin – Sulphuric Acid Reagent



Under visible light **short U.V at 254 nm** **Under U.V. at 366nm**

- Track 1: Blank (Methanol; 2 µL)
- Track 2: B-Sitosterol (Concentration 4000 ng/band)
- Track 3: Extract 4 . (Volume Applied 10 µL)
- Track 4: Extract 5. (Volume Applied 10 µL)
- Track 5: Extract 6.(Volume Applied 10 µL)
- Track 6: Extract 9.(Volume Applied 10 µL)
- Track 7: Extract 12. (Volume Applied 10 µL)
- Track 8: Extract 15 .(Volume Applied 10 µL)
- Track 9: Blank (Methanol; 2 µL)

Results1:

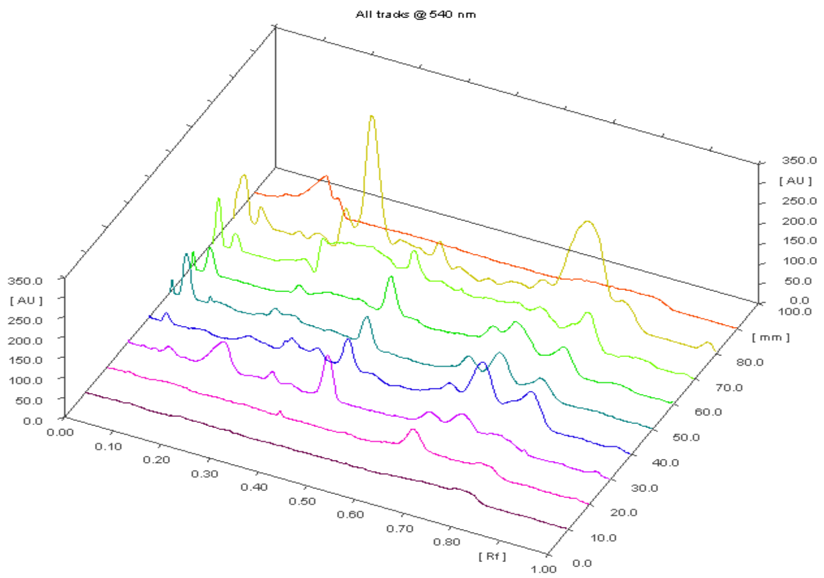


Figure A: 3D Densitogram of all tracks (Scanned @ 540 nm after derivatization)

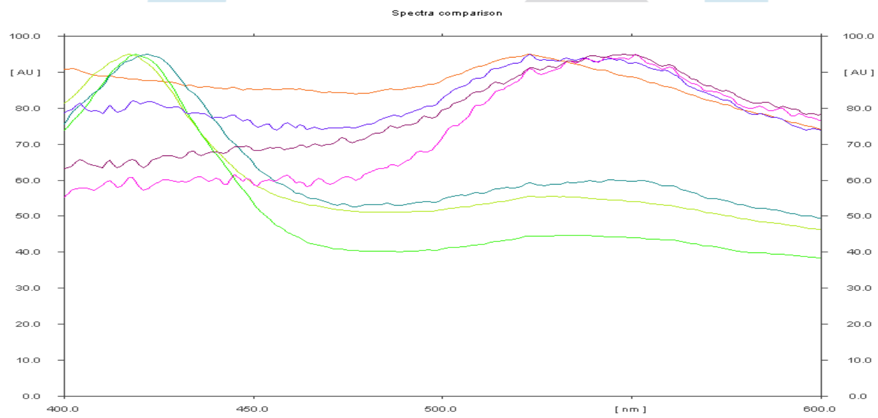


Figure: B-sitosterol (Track 2, Rf – 0.64) Spectra of Standard [Magenta] and Extract Samples = Match

Track no.	Sample name	Rf values
2	**Standard (Beta sitosterol)**	**0.64**
3	**4. *Bauhinia varigata* (Kanchanar)**	**0.62**
4	**5. *Tinospora cordifolia* (Gulvel)**	**0.62**
5	**6. *Eclipta alba* (Bhringraj)**	**0.62**
6	**9. *Moringa oleifera* (Shevga)**	**0.63**
7	***12. Cassia tora* (Tarvat)**	**0.64**

8	**15. *Citrus limon* (Limbu)**	**0.64**

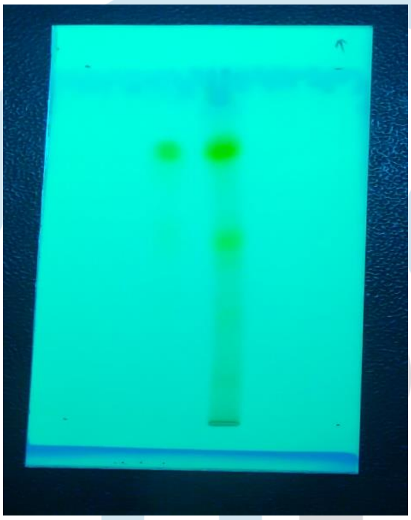
B-sitosterol present in Samples Bauhinia varigata ((Kanchnar), *Tinospora cordifolia* (Gulvel), 6. *Eclipta alba* (Bhringraj), *Moringa oleifera* (Shevga), Cassia tora (Tarvat) and Citrus limon (Limbu).

Spectra for Extract Samples 6.*Eclipta alba* (Bhringraj), 9. *Moringa oleifera* (Shevga) and 12. *Cassia tora* (Tarvat) slightly vary from standard B-sitosterol spectrum in the region 400 nm to 450 nm. That may be due to partial overlap of peaks.

Observation2:

Sample 11. Curcuma amada (Ambehalad)

Under visible light



short U.V at 254 nm

Under U.V. at 366nm

Track 1: Blank (Methanol; 2 µL)

Track 3: Extract 11. *Curcuma amada* (Volume Applied 10 µL)

Track 2: Curcumin Standard (2 µL, Concentration 2000 ng/band)

Track 4: Blank (Methanol; 2 µL)

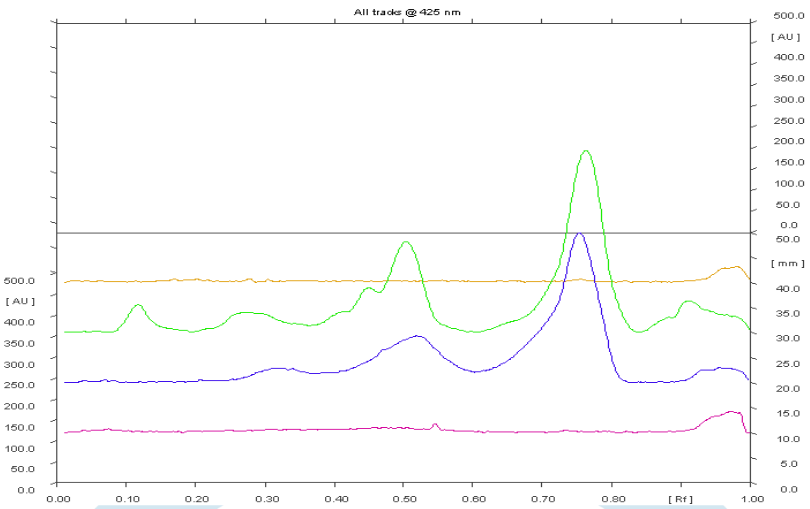


Figure 3D Densitogram of all tracks (Scanned @ 425 nm)

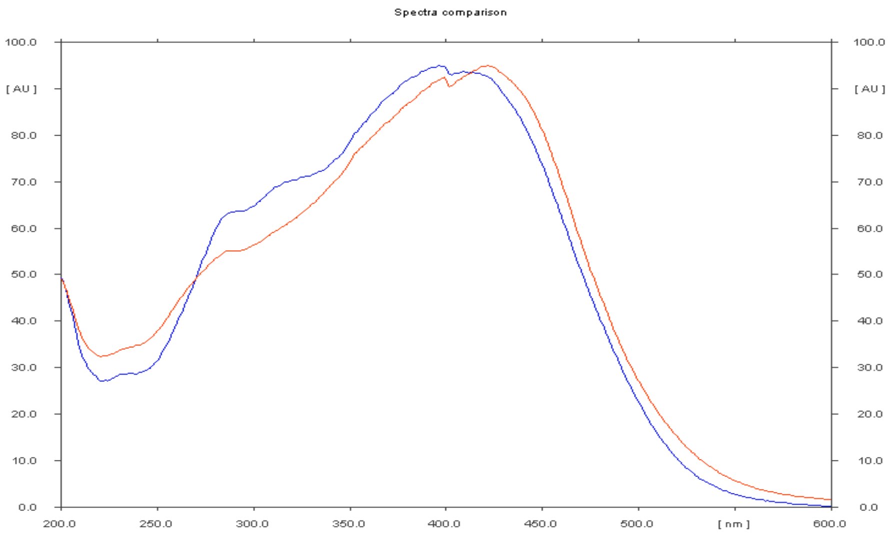
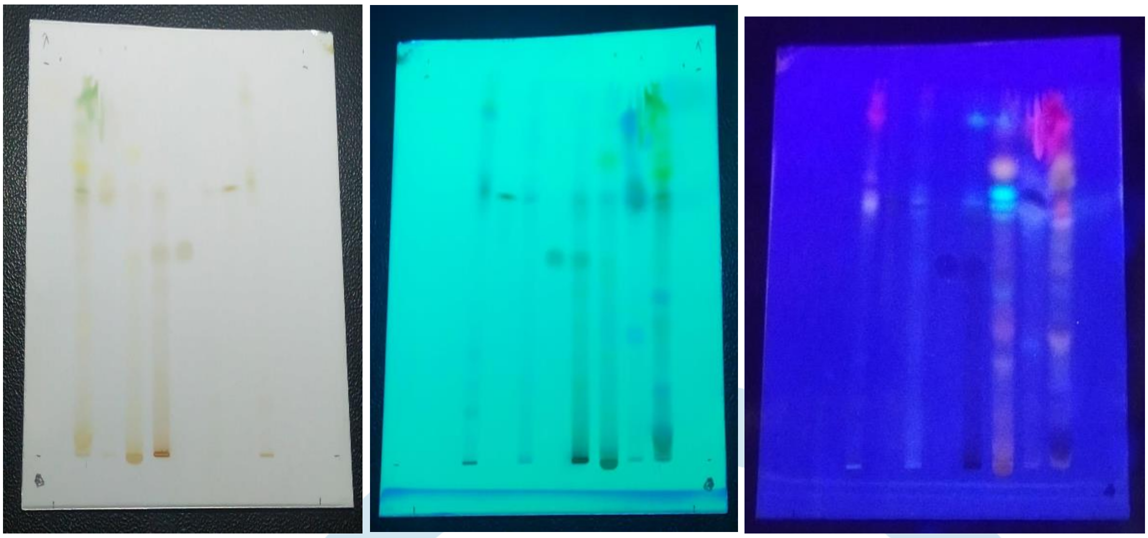


Figure: Spectra Comparison of Peak at Rf - 0.75 (Standard and Sample)

- Curcuminoids present in Sample 11. Ambehalad (*Curcuma amada)*
- Study can be further extended for quantification

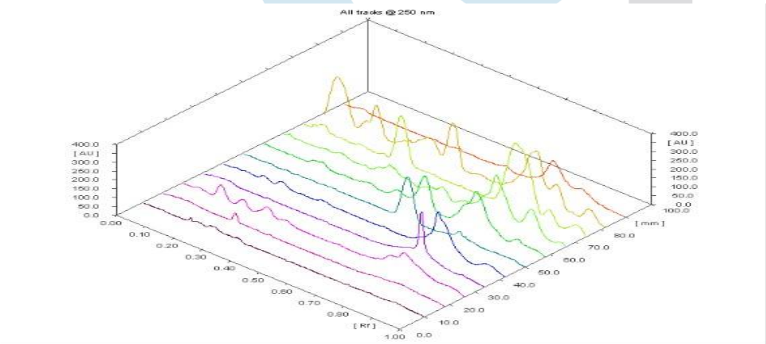
Observation 3:



Under visible light **Under U.V. at 254 nm** **short U.V at 366nm**

- Track 1: Blank (Methanol; 2 µL)
- Track 2: Ascorbic Acid Standard (Concentration 2000 ng/band) Track 3: Extract 2.Volume Applied 10 µL)
- Track 4: Quercetin Standard (Concentration 2000 ng/band) Track 5: Extract 7.(Volume Applied 10 µL)
- Track 6: Gallic Acid Standard (Concentration 2000 ng/band) Track 7: Extract 8 .(Volume Applied 10 µL)
- Track 8: Extract Sample 1. (Volume Applied 10 µL)
- Track 9: Extract Sample 3.(Volume Applied 10 µL) Track 10: Extract Sample 10. (Volume Applied 10 µL)
- Track 11: Blank (Methanol; 2 µL)

Results:



3D Densitogram of all tracks (Scanned @ 250 nm)

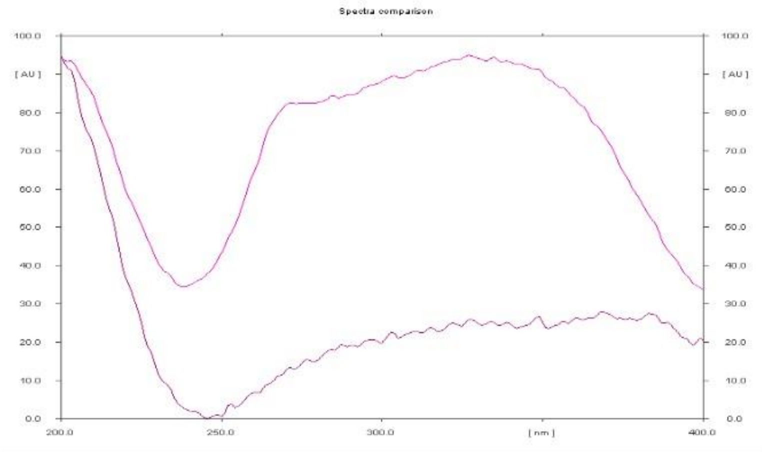


Figure: Ascorbic Acid (Track 2, Rf – 0.23) Spectra of Standardand Extract 2. Bel Track 3) = Match

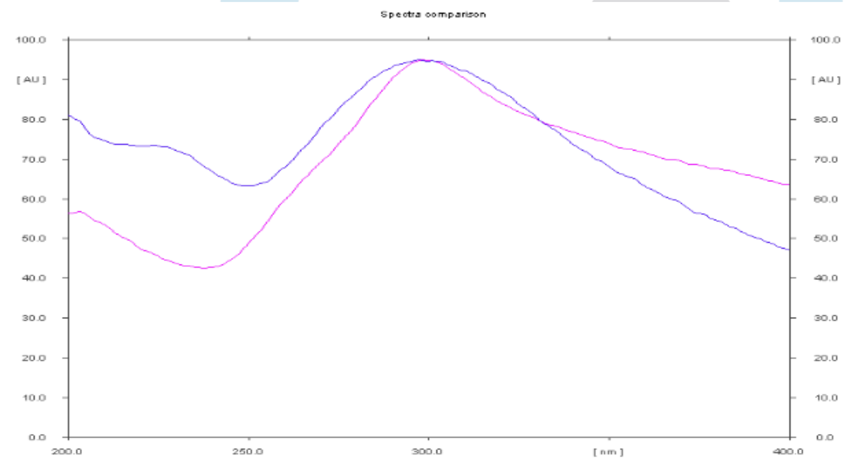


Figure: Quercetin (Track 4, Rf – 0.78) Spectra of Standard and Extract 7. *Withania somnifera* (Track 5)=Match

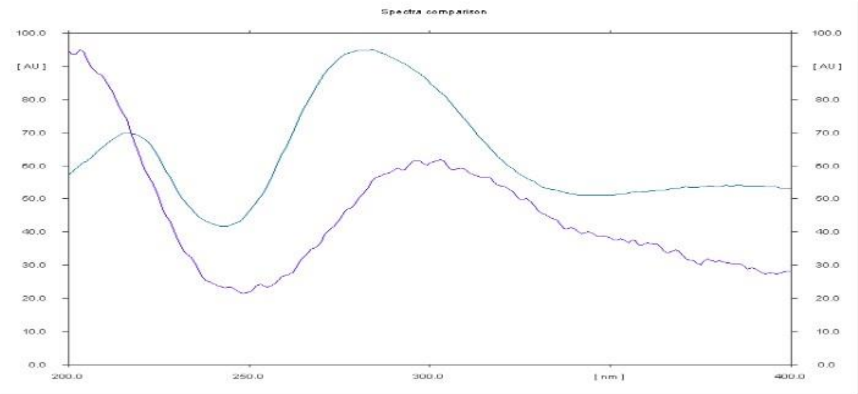


Figure: Gallic Acid (Track 6, Rf – 0.51) Spectra of Standard and Extract 8 *Azadirachta indica* (Track 7) =Match

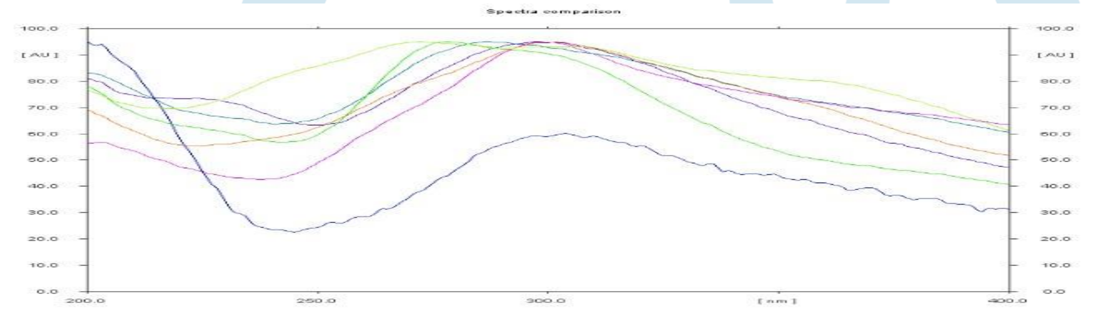


Figure: Quercetin (Track 4, Rf – 0.23) Spectra of Standard and Extract Sample7. *Withania somenifea* (Track 5) = Extract Sample1 . *Aloe vera*(Track 8) = Match Extract Sample3 . Psoralea coryfolia(Track 9) = Match Extract Sample10 Sonamukhi(Track 10) = Match

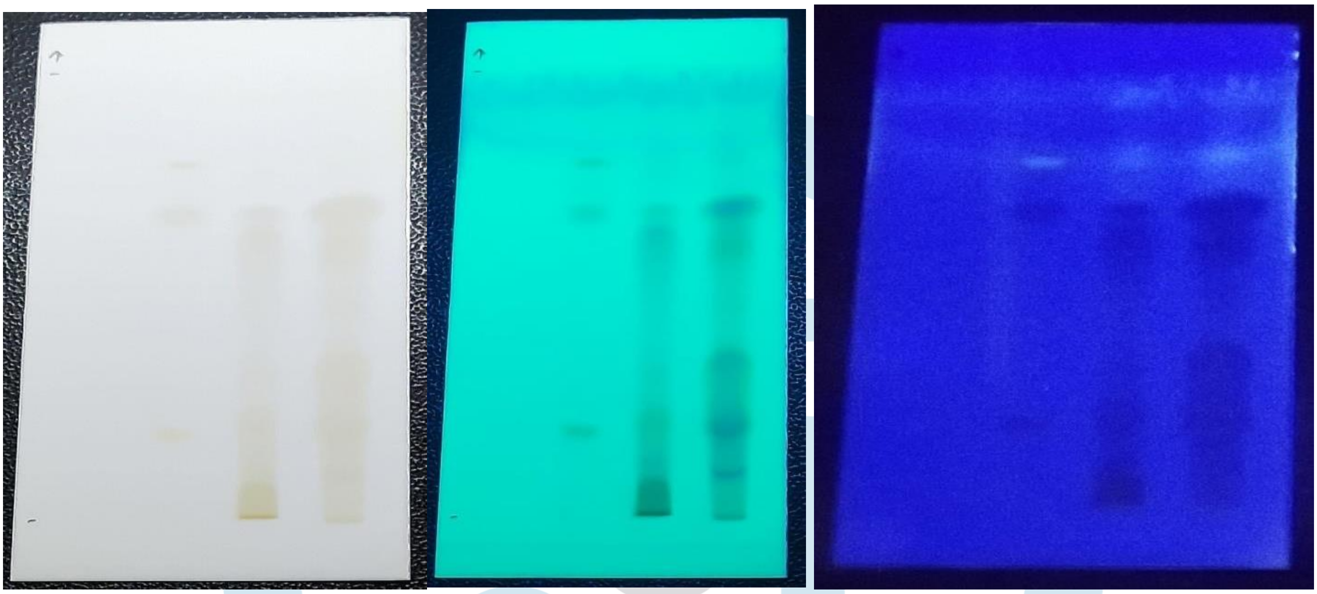
Results:

Track no.	Sample Name	Rf values
2	Ascorbic acid	0.23
3	*Aegle marmelos*	0.23
4	Quercetin	0.78
5	*Withania somnifera*	0.77
6	Gallic acid	0.51
7	*Azadirachta indica*	0.50
8	*Aloe vera*	0.76
9	*Psoralea coryfolia*	0.76
10	*Cassia agustifolia*	0.76

- **Ascorbic Acid present in Sample 2. Bel** *Aegle marmelos.*

- **Quercetin present in Samples7.** *Withania somnifera*, (**Aswagandh), 8**. *Azadirachta indica* **(Kadulimb), 1.** *Aloe vera* **(Korphad), 3.** *Psoralea coryfolia* (**Bawachi) and 10.** *Cassia agustifolia* **(Sonamukhi).**
- **Gallic Acid present in Sample8.** *Azadirachta indica*

Observation 4:



Under visible light **short U.V at 254 nm** **Under U.V. at 366nm**

Track 1: Blank (Methanol 2 µL)

Track 2: Rutin + Gallic Acid + Quercitin (Concentration 2000 ng/band each) Track 3: Extract Sample 13 (Volume Applied 10 µL)

Track 4: Extract Sample 14 (Volume Applied 10 µL)

Results:

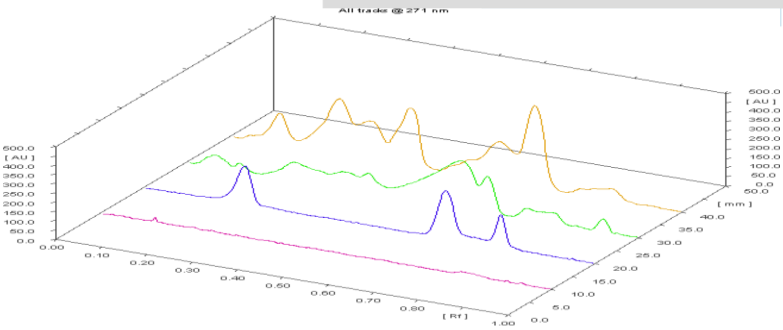


Figure: 3D Densitogram of all tracks

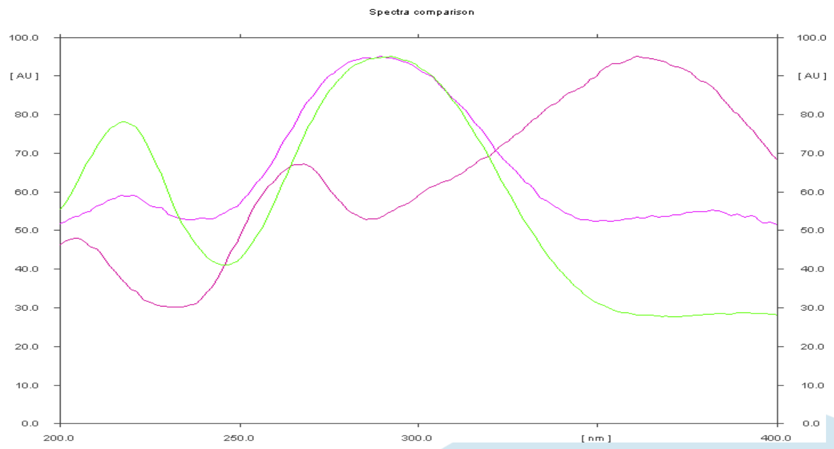


Figure: Rutin (Rf – 0.23) Spectra of Standard [Pink with 03 Peaks] and in Extract Sample 13. *Punica granatum* and 14. *Phyllanthus emblica* [Green and Pink] = Do not Match

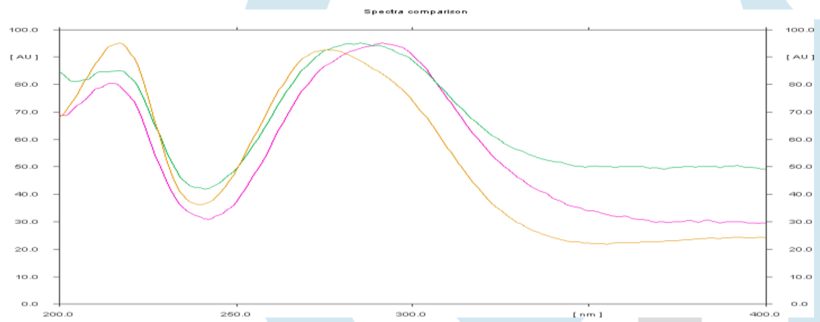


Figure: Quercetin (Rf – 0.79) Spectra of Standard [Pink with two peaks]

And in Extract Sample 13. *Punica granatum* and 14. *Phyllanthus emblica*--Do not match perfectly

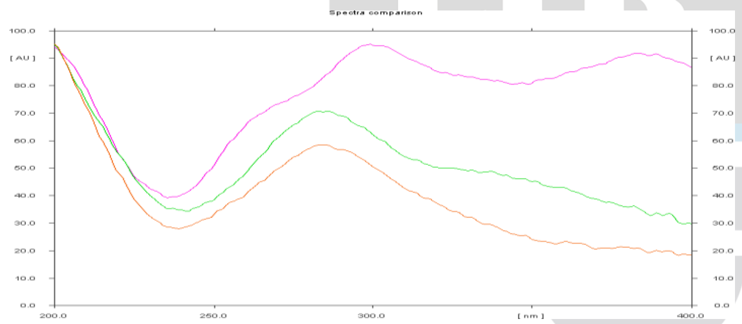


Figure: Gallic Acid (Rf – 0.79) Spectra of Standard and in Extract Sample 13 . Dalimb and 14. Amla

= Exactly Match

- Rutin Absent in both Sample 13. *Prunus granatum* and 14. *Phyllanthus emblica*.
- Gallic Acid Present in both Sample 13. *Punica granatum* and 14. *Phyllanthus emblica*.
- Quercetin may be present in both Sample 13. *Punica granatum* and 14. *Phyllanthus emblica*

Results and Discussion

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The objective of this study was to identify analysis technique using the different solvent systems for selected fifteen medicinal plants, which has been developed and validated for simultaneous quantification of six marker compounds (quercetin, rutin, gallic acid, ascorbic acid, curcumin and beta sitosterol) in plant extracts. These flavonoids have been shown to be among the active principles in some herbal extracts. HPTLC was used for qualitative and identification of flavonoids in complex plant extracts. HPTLC fingerprint analysis Four different mobile phases previously described for the separation of flavonoids were tested, using silica gel HPTLC plates, namely ethyl acetate:formic acid:water (6:1:1, v/v) (Annegowda et al. 2012), ethyl acetate:formic acid: acetic acid:water (100:11:11:26, v/v) (Wagner & Bladt 2001), ethyl acetate: methyl ethyl ketone: formic acid:water (50:30:10:10, v/v) (Polish Pharmacopoeia 2002), and ethyl acetate:formic acid:water (82:9:9, v/v) (European Pharmacopoeia 2007).

Conclusion

This newly developed and validated HPTLC method provides a new approach to estimate major plant flavonoids like quercetin, rutin, gallic acid, beta sitosterol, curcumin and ascorbic acid in extract of plants like *Aegle marmelos, Aloe vera, Eclipta alba, Punica granatum, and Psoralea coryfolia, Bauhinia variegata, Tinospora cordifolia, Moringa oleifera, Azadirachta indica, Withania somenifera, Cassia tora, ,Curcuma amada, Cassia angustifolia, Emblica officinalis, Citrus limon.* The method was found to be simple, precise, specific, sensitive, efficient, and easy to use for simultaneous analysis of quercetin, rutin, gallic acid, beta sitosterol, curcumin and ascorbic acid .It can also be used in routine quality control of herbal materials as well as formulations containing any or all of these compounds. The validated method renders it useful for both analytical research and for routine analysis in the plant crude extracts for their quality control.

References

[1] Mirghani MES, Liyana Y, Parveen J. 2012. Bioactivity analysis of lemongrass Cymbopogan citratus essential oil. Int. Food Res J. 192:569–575.

[2] Annegowda HV, Mordi MN, Ramanathan S, Hamdan MR, Mansor SM. 2012. Effect of extraction techniques on phenolic content, antioxidant and antimicrobial activity of Bauhinia perpura, determination of antioxidants. Food Anal Methods. 5:226–233.

[3] Pereira CAM, Yariwake JH, Lanças FM, Wauters JN, Monique T, Angenot L. 2004. A HPTLC densitometric determination of flavonoids from Passiflora alata, P. edulis, P. incarnata and P. caerulea and comparison with HPLC method. Phytochem Analy. 15:241–248.

[4] Wu X, An Y, Yuan M, Wang R. 2012. On the modernization of traditional Chinese medicine. Med Aromat Plants. 1:e105

[5] Karunamoorthi K, Jegajeevanram K, Jegajeevanram V, Mengistie E. 2013. Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. J. Evid. Based Compl. Alternative Med. 18:67–74. Khare CP. 2012. The modern ayurveda: milestones beyond the

[6] Nile SH, Park SW. 2014a. HPTLC analysis, antioxidant, anti-inflammatory and antiproliferative activities of Arisaema tortuosum tuber extract. Pharm. Biol. 52:221–227.

[7] Nile SH, Park SW. 2014b. HPTLC analysis, antioxidant and antigout activity of Indian plants. Iranian J Pharmaceutical Res. 12:531–539.

[8] Nile SH, Khobragade CN. 2010. Studies on antioxidant activity and inhibitory potency of plants based flavonoids on xanthine oxidase. Ph.D. Thesis in Biotechnology, Faculty of Science, Swami Ramanand Teerth Marathwada University, Nanded (MS), India

[9] Polish Pharmacopoeia VI. 2002. Polish Pharmaceutical Society, Warsaw.

[10] European Pharmacopoeia. 2007. 6th edn. Council of Europe, Strasbourg. Glavnik V, Simonovska B, Vovk I. 2009. Densitometric determination of +.−catechin and −.−epicatechin by 4- dimethylaminocinnamaldehyde reagent. J. Chromatogr. A. 1216:4485–4491.

[11] Nile SH, Park SW. 2014. HPTLC densitometry method for simultaneous determination of flavonoids in selected medicinal plants. Frontiers in Life Science, 2015 Vol. 8, No. 1, 97–103.

[12] Wankhade M.S., Mulani R.M., 2015.Chromatography Finger print profiling and phytochemical investigation on leaf and bark methanolic extract Ougeinia oojeinesis (ROXB). HOCHR. International Journal of Current Research Vol. 7, Issue, 02, pp.12665-12673.

[13] Thomas A., Kanakdhar A., Shirsat A., Dheshkar S., Kothapalli L. A High Performance Thin Layer Chromatographic Method Using a Design of Experiment Approach for Estimation of Phytochemicals in Extracts of Moringa Oleifera Leaves. Turk J Pharm Sci 2020; 17(2):148-158.

[14] Amir M, Mujeeb M, Ahmad S, Akhtar M, Ashraf K. Design expert supported development and validation of HPTLC method: an application in simultaneous estimation of quercetin and rutin in Punica granatum, Tamarindus indica and Prunus domestica. Pharm Methods. 2013; 4:62-67.

[15] Sajeeth CI, Manna PK, Manavalan R, Jolly CI. Quantitative estimation of gallic acid, rutin and quercetin in certain herbal plants by HPTLC method. Der Chemica Sinica. 2010; 1:80-85.

[16] Singh, R., Singh, S.K., Arora, S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn. Food Chem. Toxicol., 45: 1216-1223.2007.

[17] Mohammad A. Alzohairy, Therapeutics Role of Azadirachta indica (Neem) and their Active constituents in Diseases Prevention and Treatment. Disease. 2016.

[18] Rathi R., Chopra B. Withania somnifera, naturally occurring chemicals AgainstAlzheimer's.2021.

[19] Pawar and D'mello, IJPSR, CASSIA TORA LINN.: AN OVERVIEW Vol. 2(9): 2286-2291 ISSN: 0975-8232. 2011.
[20] Ravindran P.N., Divakaran M., in Handbook of Herbs and Spices (Second Edition), Volume 2, 2012
[21] Manjeshwar S.B., Rao S., Hepatoprotective Effects of the Indian Gooseberry (Emblica officinalis Gaertn), in Dietary Interventions in Liver Disease.2019.
[22] Alexandra Mara G. N. M, Antonio G. S., Lemon, in Nutritional Composition and Antioxidant Properties of Fruits and Vegetables.2020.