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Original Research Article

Validated high performance thin layer chromatographic analysis of leaves and flower extracts of *catharanthus roseus*Harshita Tripathi¹, Anup K Chakraborty^{1,*}, Sarita Karole¹, Kavita R Loksh¹¹Dept. of Pharmaceutical Chemistry, Oriental College of Pharmacy, Bhopal, Madhya Pradesh, India

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ABSTRACT

Background: *Catharanthus roseus* is a significant therapeutic plant has a place with family apocynaceae contains in excess of 70 distinct sorts of chemotherapeutic agents and alkaloids which help in treating different illnesses. For the most part, it is known as Vincarosea, Ammocallisrosea and Lochnerarosea. There are numerous or more than 400 alkaloids present in plant, which are used as flavor, agrochemicals, pharmaceuticals, fragrance, ingredients, food additives, and pesticides.

Aims and Objectives: To develop a validated high performance thin layer chromatographic method for the analysis of leaves and flower extracts of *Catharanthus roseus*.

Materials and Methods: Sample solutions were applied onto the plates with automatic TLC sampler Linomat V (Camag, Muttentz, Switzerland) and were controlled by WinCATS software. Plates were developed in 10 x 10cm twin trough glass chamber (Camag, Muttentz, Switzerland). A CAMAG TLC scanner was used for scanning the TLC plates. Pre-coated silica gel aluminium plates 60F254.

Results: For vincristine, simultaneous estimation of vincristine was performed by HPTLC on a silica gel plate using toluene-methanol-diethylamine (8.75: 0.75: 0.5, v/v/v) as the mobile phase. The method was validated as per the ICH guidelines. The Rf value was found to be 0.76 for flower and 0.80 for leaves at 250 nm which shows the presence of vincristin in *Catharanthus roseus*.

Conclusion: In this research paper, a validated HPTLC Method has been developed for the analysis of *Catharanthus roseus* leaves and flower extracts

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1. Introduction

Mainly plants are used for variety of diseases related to various treatments. The are several secondary metabolites plants produce including alkaloids, flavonoids, steroids, Saponins, glycosides and Terpenoids to protect them from various outside metabolites or natural occurring pathogens like insects, pests and various environmental stresses.

Catharanthus roseus is an evergreen sub spice plant of 1m tall. The leaves of the plant are oval to elongated, 2.5-9.5 cm long and 1-3.5 cm wide with a reflexive green bear with a short petiole around 1-1.8 cm long and pale midrib

organized in a opposite pair. The flowers are dark pink to white with a dull red place with a basal cylinder about 2.5-3cm long and corolla around 2-5cm width with petals like projections. The fruit is a couple of fol licles around 2-4cm long and 3mm wide.¹

Catharanthus roseus is a significant therapeutic plant has a place with family apocynaceae contains in excess of 70 distinct sorts of chemotherapeutic agents and alkaloids which help in treating different illnesses. For the most part, it is known as *Vinca rosea*, *Ammocallis rosea* and *Lochnera rosea*.²

Catharanthus roseus are developed by two regular names, in view of their flower colour, pink-rosea, white-alba. The leaves of *Catharanthus roseus* are utilized as

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a medication for the treatment of different infections like acid reflux, feminine problem, stiffness, dyspepsia, diabetes and have antiviral and narcotic properties. The leaves of the plant have more than 70 types of chemical constituents such as serpentine and reserpine, indole type of alkaloids, ajmalicine. Due to presence of alkaloids, it has antispasmodic and antihypertensive properties. *Catharanthus roseus* produce important type of alkaloid which is Vinblastine due to its wide pharmaceutical use and antitumor property. It is also used to produce chemotherapeutic agents for their pain-relieving properties. It also acts as a wound healer. Most medicinal plants have wide range or variety of natural oxidants like Flavanoids, tannins and phenolic than dietary plants. *Catharanthus roseus* contains remarkable amount of phenolic and volatile compounds including caffeoyl quinic acids and flavonol glycosides which are known for antioxidant property. It also plays a significant role in the body defense system as it acts as an antioxidant against reactive oxygen species, which are harmful by forming such products through normal aerobic respiration. Accumulation or scattering of free radical cause pathological condition such as asthma, arthritis, ageing process, inflammation, neuro degeneration and perhaps dementia.³

The petals, seeds and other parts exhibit antioxidant property. Thus, the phenolic compound present in plant have redox properties acts as hydrogen donor, metal chelators or singlet oxygen quenchers and reducing agents. It has many applications in cosmetics, food and pharmaceutical industries. These compounds also exhibit various properties other than antioxidant property like anti-inflammatory, anti-thrombotic, anti-allergic, anti-microbial, vasodilator effect and cardio protective property. This is influenced by geographical and environmental factors. Natural oxidant as a source of finding effective, cheap and safe oxidant. These are collectively known as free radical scavenger. This type of oxidants is mainly applied to avoid or to prevent lipid oxidation in the food industries.⁴



Fig. 1: Leaves and flowers of *Catharanthus roseus*

1.1. Active chemical constituents

The medicinal properties investigated by researchers discovered that it contained different types or a group of alkaloids that are toxic and can be used only in the treatment of cancer. But plants have the capacity to synthesize various chemicals that can be used for biological system and also used to protect plant from predators like insects, fungi and herbivorous animals. Alkaloids are the most active compound of the plant *Catharanthus roseus*. There are numerous or more than 400 alkaloids present in plant, which are used as flavor, agrochemicals, pharmaceuticals, fragrance, ingredients, food additives, and pesticides. The alkaloids present mainly in aerial parts are Vinblastine, Vincristine, Vindeline Tabersonine, actineo plastidemic, Vindesine, etc, whereas ajmalicine, catharanthine, vineamine, reserpine, vinceine, raubasin, etc are present in basal stem and roots. The anthocyanin pigment found in the flower of *Catharanthus roseus*.⁴

1.2. Pharmacological activities

As per the literature survey over *Catharanthus roseus*, it is found that majority of parts of this plant possess effective medicinal properties⁵ like Anti — diabetic, anti cancer, anti microbial,⁶ hypolipidimic,⁷ anti oxidant, anti ulcer etc.

2. Materials and Methods

Collection and processing of plant the *Catharanthus roseus* has been collected from areas of Bhopal city the plant was collected and dried in the shade. Finally, the mixture was powdered.

2.1. Extraction process for catharanthus roseus

Extraction of plant material root flower and leaves nearly 50 grams of plant materials leaves roots flowers weigh separately and accurately and the extraction process was carried out using cold maceration. Only 2 solvents have been used that is petroleum ether and methanol.

2.2. Qualitative analysis of phytochemicals from leaves extract

2.2.1. Detection of alkaloids

The concentrates is broken down in dil. HCl and afterwards filter. The filtrate was then additionally treated or tried with various reagents to recognize the presence of alkaloids.

1. *Dragendroff's test:* Filtrate was treated with potassium bismuth iodine solution (Dragendroff's reagent). Formation of orange red encourage demonstrated the presence of alkaloids.
2. *Hager's test:* Filtrate was treated with soaked watery arrangement of picric acid (Hagers' reagent)

Presence of alkaloids were affirmed by the development of yellow shaded encourage.

3. *Mayer's Test*: Filtrate was treated with Mayer's reagent (potassium mercuric iodide arrangement). Formation of cream shaded or whitish yellow encourage demonstrates the presence of alkaloids.

2.2.2. Detection of carbohydrates- dissolve 2g concentrate in 5ml refined water and afterward filter it

- The filtrates were utilized to test for the presence of carbohydrate.

a) *Molish's test*: Filtrate was treated with 2 drops of alcoholic alpha-naphthol arrangement in a test tube at that point shaken.

1. Add conc. Sulphuric acid from the side of the test tube.
2. Development or presence of a violet ring at the intersection of two fluid affirmed the presence of sugars.

2.2.3. Detection of reducing sugars

a) *Benedict's Test*: Filtrate was treated with Benedict's reagent and bubble in thermostatic water shower for 5 minutes.

- Formation of an orange red accelerate showed or decided the presence of lessening sugars.

b) *Fehling's Test*: filtrate was fermented with dil. HCl, killed with soluble base and afterward warmed with Fehling's A and B arrangements.

- Formation of red precipitate showed the presence of lessening sugars.

2.2.4. Detection of saponins

1. *Froth Test*: Extract was weakened with refined water to 20 ml and shaken for 15 minutes in a graduated test tube.

- Formation of layer of 1cm froth showed the presence of Saponins.

2. *Foam Test*: Small measure of the concentrate was taken with 2ml of water and shaken.

- Persistence of froth created for 10 minutes demonstrated the presence of Saponins

2.2.5. Detection of phytosterols — small amount of concentrate broke up in 5ml of chloroform

a) *Salkowski's test*: On adding a couple of drops of conc. Sulphuric corrosive.

1. Permit the solution for stand
2. Arrangement of earthy colored ring demonstrates the presence of phytosterols.

b) *Libermann burchard's test*: The chloroform separated was treated with not many drops of acidic anhydride.

1. Boil and cool.
2. Add conc. Sulphuric acid.
3. Development of pale blue green shading arrangement affirmed the presence of phytosterols.

2.2.6. Detection of phenolic compounds

1. *Ferric chloride test*: Treat the concentrate with 3-4 drops of ferric chloride arrangement. Development of somewhat blue dark shading showed the presence of phenols.

2. *Lead acetic acid derivation test*: Treat the concentrate with 3ml of 10% lead acetic acid derivation arrangement.

- A cumbersome white hasten shows the presence of phenolic compounds.

2.2.7. Detection of tannins-take 0.5g of the dried powder plant

1. Boiled 0.5g example in 20ml of water in a test tube.

2. Filter the above blend.

3. Add not many drops of 0.1% ferric chloride.

4. Advancement of an earthy green or a blue dark coloration showed the presence of tannins.

2.2.8. Detection of flavonoids

1. *Lead acetic acid derivation test*: Treat the concentrate with not many drops of lead acetic acid derivation arrangement.

- Arrangement of yellow encourage showed the presence of flavonoids.

2. *Alkaline reagent test*: treat the concentrate with not many drops of sodium hydroxide arrangement.

- Development of extraordinary yellow tone, which gets dry on additional option of weaken acid, shown the presence of flavonoids.

2.2.9. Detection of proteins and amino acids

1. *Ninhydrin test*: Add Ninhydrin reagent to the test arrangement and boiled for few moments.

- Development of blue shading demonstrated the presence of amino acids.

2. *Biuret's test*: Treat the test arrangements with few drops of 2% of copper sulfate arrangement.

- Add 1 ml of ethanol followed by abundance of potassium hydroxide pellets.
- Arrangement of pink tone in the concentrate layer shows the presence of Pr.

3. *Millon's test*: Treat the arrangement with few drops of Millon's reagents.

- At the point when warmed a white hasten is framed which changes to a block red or vanishes; shows the presence of protein and amino acids.

2.2.10. Detection of terpenoids

1. *Salkowski's test*: blend 2ml of chloroform to separate solution, carefully added conc. Sulphuric acid (3ml) to shape a layer.

- A rosy earthy colored coloration of the interface showed the presence of Terpenoids.

2.2.11. Detection of cardiac glycosides

1. *Keller-Killani Test*: Treat the extract with 2ml of glacial acetic acid with one drop of ferric chloride solution.
2. Now add 1ml of conc. Sulphuric acid.
3. Appearance of brown ring at the interface indicates the deoxy sugar characteristics.
4. Appearance of a violet ring below the brown ring and a greenish ring in the acetic acid layer confirmed the presence of cardiac glycoside.

2.3. Percentage yield

The plant material was extracted by cold maceration and the percentage yield calculated by the formula $\text{Yield (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$ was found to be 0.564 (petroleum ether) and 2.818 (methanol).

2.4. Physical parameters of extracts of *catharanthus roseus*

Morphological character find out by observing Colour, texture, smell and taste

2.5. Total phenolic concentration (TPC of extract of *catharanthus roseus*)

2.5.1. Spectrophotometric quantification of total phenolic content

Procedure: The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10fold dilute folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at

room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically.

2.6. Total flavonoid content of extract of *Catharanthus roseus*

2.6.1. Spectrophotometric quantification of total flavonoid content

Total flavonoids were measured by a colorimetric assay according to Dewanto et al. An aliquot of diluted sample or standard solution of rutin was added to a 75 μl of NaNO_2 solution, and mixed for 6 min, before adding 0.15 mL AlCl_3 (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. All samples were analyzed in three replications.

2.7. Percentage yield of extract of flowers

The plant material was extracted by cold maceration and the percentage yield calculated by the formula $\text{Yield (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$ was found to be 0.023 (petroleum ether) and 0.456 (methanol).

Note: Phytochemical tests, Phenolic concentration, Flavonoid concentration are find out by same procedure follow for leaves extract.

2.8. HPTLC (High performance thin layer chromatography)

HPTLC is an analytical technique or method used for quantitative and qualitative evaluation of polyherbal formulation. *Catharanthus roseus* is an important medicinal plant used in large number of herbal preparations.

2.9. Chromatographic conditions

Sample solutions were applied onto the plates with automatic TLC sampler Linomat V (Camag, Muttenz, Switzerland) and were controlled by WinCATS software. Plates were developed in 10 x 10cm twin trough glass chamber (Camag, Muttenz, Switzerland). A CAMAG TLC scanner was used for scanning the TLC plates. Pre-coated silica gel aluminium plates 60F254. The plates were pre-washed with methanol and initiated at 60°C for 5minutes before chromatography. Six various aliquots (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 4.0 μL) of standard arrangement were applied

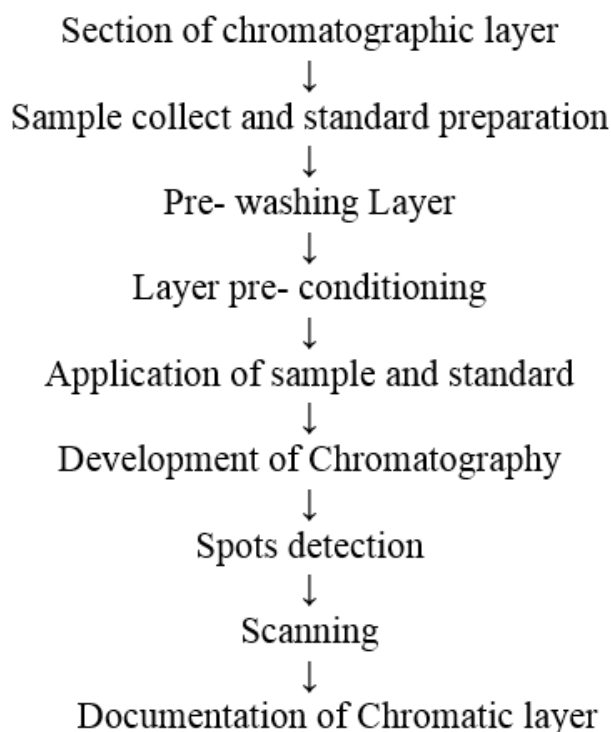


Fig. 2: Process for HPTLC Chromatogram

on 10 x 10 cm TLC plates for the planning of alignment bend. A steady application pace of 150 nL/s was employed. The cut measurement was kept at 4.0 x 0.30 mm and filtering velocity of 20 mm/s was employed. 20 mL of portable stage comprised of toluene-diethylamine-methanol (7.3:2.5:2 v/v/v) was utilized per plate. The plates were created and filtered inside 10 min utilizing densitometry scanner III in the remission mode at 307nm for vincristine. Assessment was finished by measuring peaks with linear regression.

2.10. Preparation of standard solutions

Standard arrangements of vincristine and were set up by dissolving 10mg of vincristine in 10ml of methanol (1000 µg/ml). This stock arrangement was utilized to make calibration bend of vincristine.

2.11. Preparation of sample solutions

Weighed 50gm of *Catharanthus roseus* leaves also, boiled it for 2 hrs. on an electric water bath. Powder the leaves and afterward blended it with adequate amount of alcoholic KOH and dried the powder in oven at 100°C. A precisely weighed quantity (2 g) of leaves were sonicated for 20 minutes in 4ml. of methanol independently. The arrangements were sifted and gathered in vials, extracted the drug with 150 ml. methanol in soxhlet device for 6 hrs. Methanol remove was isolated and shaken with progressive

three parts of 5ml.dilute sulphuric corrosive. Combine the acid extract and afterward separated. Add excessive amount of ammonia to the acid concentrate extract to precipitate the alkaloids and filter. Dry the precipitate and then weighed. The precipitate was then mixed or dissolved in methanol (200mg/ml).

3. Results and Discussion

3.1. Qualitative phytochemical analysis of leave extracts

| S. No. | Experiment | Result | |
|---|--------------------------|-------------------|--------------------|
| | | Pet ether extract | Methanolic extract |
| 1. | Molisch's Test | -ve | +ve |
| 2. | Fehling's Test | -ve | -ve |
| 3. | Benedict's Test | -ve | +ve |
| 4. | Barfoed's Test | -ve | |
| Test for protein and amino acid | | | |
| 1. | Biuret Test | +ve | -ve |
| 2. | Millons Test | -ve | +ve |
| 3. | Ninhydrin Test | -ve | +ve |
| Test for alkaloids | | | |
| 1. | Dragendorff's Test | +ve | +ve |
| 2. | Wagner's Test | -ve | -ve |
| 3. | Mayer's Test | +ve | +ve |
| 4. | Hager's Test | -ve | +ve |
| Test for triterpenoids and steroids | | | |
| 1. | Liebermann-Burchard Test | -ve | +ve |
| 2. | Salkowski Test: | -ve | +ve |
| Test for saponins | | | |
| 1. | Froth Test | -ve | +ve |
| Test for tannin and phenolic compounds | | | |
| 1. | Ferric Chloride Test | +ve | -ve |
| 2. | Gelatin Test | +ve | +ve |
| 3. | Lead Acetate Test | -ve | -ve |
| 4. | Dilute Iodine | -ve | +ve |
| Test for flavonoids | | | |
| 1. | Shinoda's Test | +ve | +ve |
| 2. | Lead acetate | -ve | +ve |
| 3. | Alkaline Test | -ve | -ve |
| Test for glycosides | | | |
| 1. | Borntragers Test | +ve | +ve |
| 2. | Keller Killiani Test | -ve | -ve |
| 3. | Legal Test | +ve | +ve |

+ve = Present; -ve = Absent

| S. No. | Solvent | % Yield |
|--------|------------|---------|
| 1. | Pet. Ether | 2.56% |
| 2. | Methanol | 12.8% |

| Extract | Colour | Texture | Taste | Smell |
|-----------|------------|------------|--------|---------|
| Pet ether | Dark Green | Semi solid | Bitter | Pungent |
| Methanol | Dark Green | Semi solid | Bitter | Pungent |

Table 1: Total phenolic concentration

| TPC Expressed as mg/gm Gallic Acid Equivalent | |
|---|---------------------------------------|
| S. No. | Methanolic Extract |
| 1 | 0.210 |
| 2 | 0.209 |
| 3 | 0.211 |
| Mean | 0.21 |
| Absorbance | |
| TPC value | 0.210.mg/gm equivalent to Gallic acid |

Table 2: Total flavonoid content

| TFC Expressed as mg/gm Rutin Equivalent | |
|---|--------------------------------|
| S. No. | MEOH |
| 1 | 0.762 |
| 2 | 0.761 |
| 3 | 0.763 |
| Mean Absorbance | 0.761 |
| TFC value | 0.76.mg/gm equivalent of Rutin |

Table 3: Yield of crude extracts of flower of *Catharanthus roseus*

| S. No. | Solvent | % Yield |
|--------|------------|---------|
| 1. | Pet. Ether | 0.23% |
| 2. | Methanol | 4.56% |

Table 4: Physical parameters of extracts of *Catharanthus roseus*

| Extract | Colour | Texture | Taste | Smell |
|-----------|-------------|------------|--------|---------|
| Pet ether | Light Green | Semi solid | Bitter | Pungent |
| Methanol | Light Green | Semi solid | Bitter | Pungent |

Table 5: Solubility determination of extract in different solvents

| S. No. | Solvent | Pet. Ether | Methanol |
|--------|-----------------|------------|-----------|
| 1. | Pet ether | Soluble | Insoluble |
| 2. | Ethyl acetate | Soluble | Soluble |
| 3. | Methanol | Insoluble | Soluble |
| 4. | Ethanol | Insoluble | Soluble |
| 5. | Distilled water | Insoluble | Soluble |
| 6. | DMSO | Soluble | Soluble |

3.2. Yield of crude extracts of *catharanthus roseus*

3.3. Physical parameters of extracts of *Catharanthus roseus*

3.4. Qualitative phytochemical analyses of flower extracts

| S. No. | Experiment | Result | |
|---|-------------------------|-------------------|--------------------|
| | | Pet Ether Extract | Methanolic Extract |
| Test for Carbohydrates | | | |
| 1. | Molisch's Test | -ve | +ve |
| 2. | Fehling's Test | +ve | -ve |
| 3. | Benedict's Test | -ve | +ve |
| 4. | Barfoed's Test | +ve | -ve |
| Test for protein and amino acid | | | |
| 1. | Biuret Test | -ve | -ve |
| 2. | Millons Test | -ve | +ve |
| 3. | Ninhydrin Test | +ve | +ve |
| Test for Alkaloids | | | |
| 1. | Dragendorff's Test | +ve | +ve |
| 2. | Wagner's Test | +ve | -ve |
| 3. | Mayer's Test | -ve | +ve |
| 4. | Hager's Test | -ve | +ve |
| Test for Triterpenoids and Steroids | | | |
| 1. | Libermann-Burchard Test | +ve | -ve |
| 2. | Salkowski Test: | -ve | -ve |
| Test for Saponins | | | |
| 1. | Froth Test | +ve | +ve |
| Test for Tannin and Phenolic Compounds | | | |
| 1. | Ferric Chloride Test | +ve | -ve |
| 2. | Gelatin Test | +ve | +ve |
| 3. | Lead Acetate Test | +ve | +ve |
| Test for Flavonoids | | | |
| 1. | Shinoda's Test | -ve | +ve |
| 2. | Lead acetate | +ve | +ve |
| 3. | Alkaline Test | -ve | +ve |
| Test for Glycosides | | | |
| 1. | Borntragers Test | +ve | +ve |
| 2. | Keller Killiani Test | -ve | -ve |
| 3. | Legal Test | +ve | +ve |

+ve = Present; -ve = Absent;

Table 6: Total phenolic concentration (TPC) of extract *Catharanthus roseus*

| TPC Expressed as mg/gm Gallic Acid Equivalent | |
|---|---------------------------------------|
| S. No. | Methanolic Extract |
| 1 | 0.268 |
| 2 | 0.264 |
| 3 | 0.272 |
| Mean Absorbance | 0.268 |
| TPC value | 0.268.mg/gm equivalent to gallic acid |

Table 7: Total flavonoid content of extract of *Catharanthus roseus*

| TFC Expressed as mg/gm Rutin Equivalent | |
|---|---------------------------------|
| S. No. | MEOH |
| 1 | 0.794 |
| 2 | 0.792 |
| 3 | 0.793 |
| Mean Absorbance | 0.793 |
| TFC value | 0.793 mg/gm equivalent of Rutin |

3.5. HPTLC chromatograms of extracts of *Catharanthus roseus*

HPTLC analysis was done using Methanol: Toluene: Diethyl amine in the ratio 7.3:2.5:2 as a solvent system. CAMAG Linomat 5 is used to develop the chromatograms

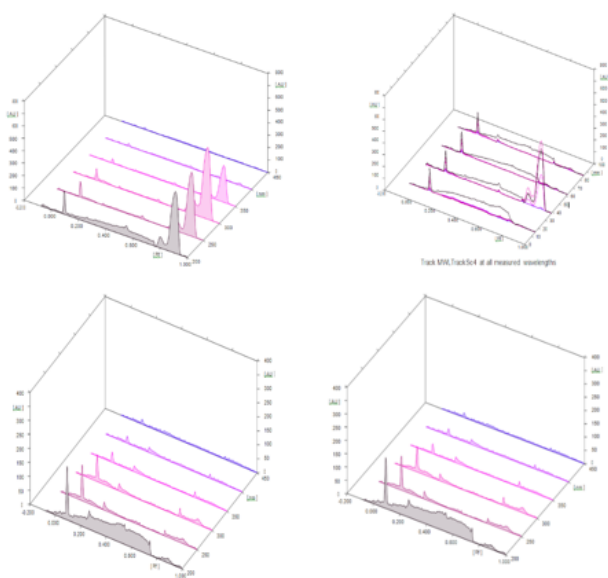


Fig. 3: All tracks at all measured wavelength

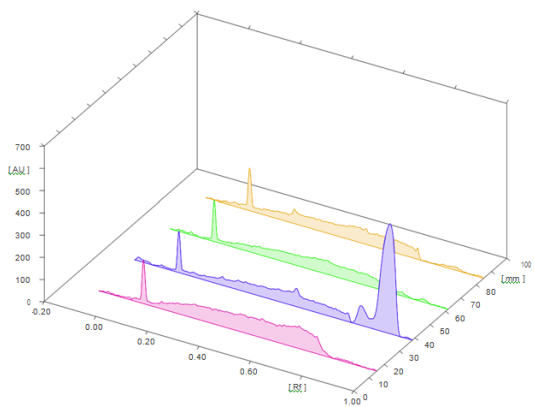
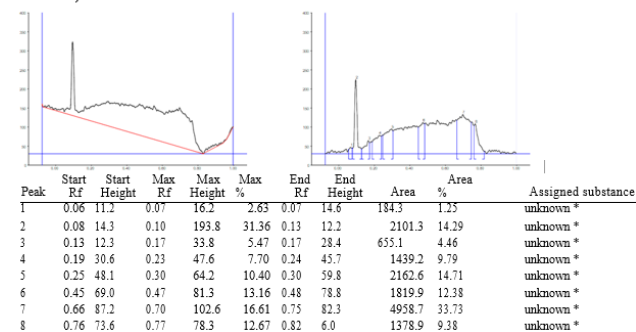
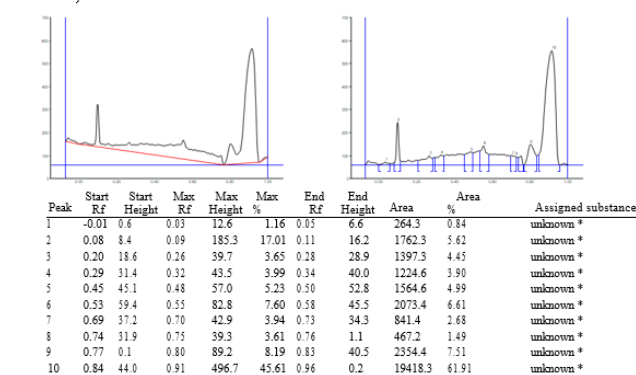


Fig. 4: All tracks at wavelength 200nm

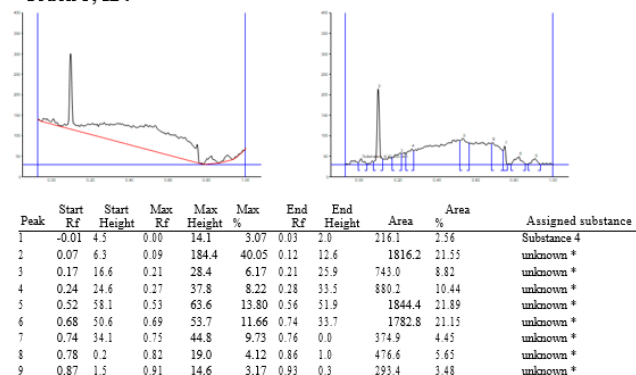
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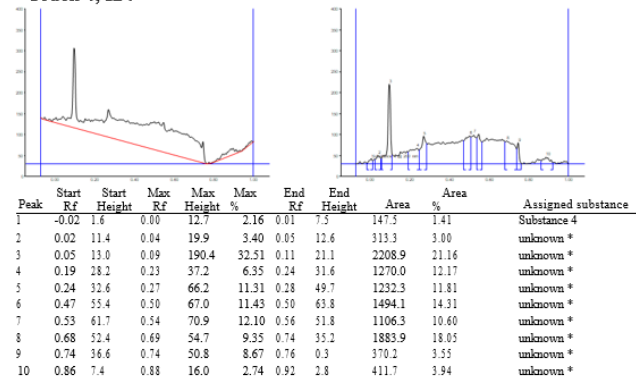
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Track 3, ID:



Track 4, ID:



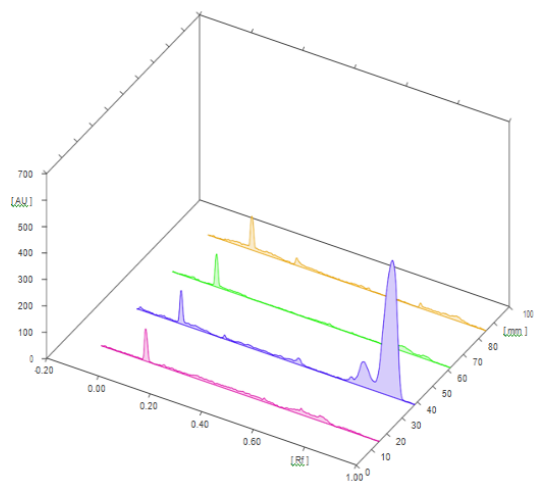
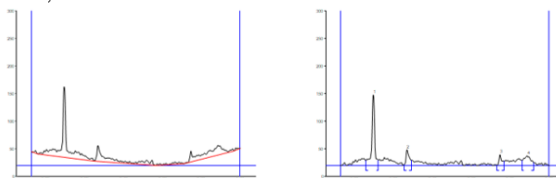


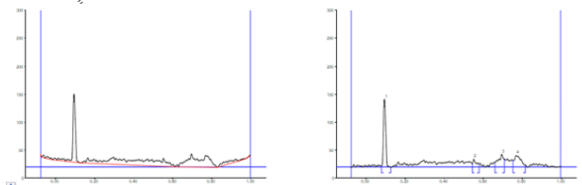
Fig. 5: All tracks at wavelength 250 nm

Track 4, ID:



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|--------|--------|--------------------|
| 1 | 0.06 | 9.1 | 0.09 | 128.1 | 66.04 | 0.12 | 9.3 | 1466.2 | 58.34 | unknown * |
| 2 | 0.25 | 1.6 | 0.27 | 28.8 | 14.82 | 0.29 | 9.4 | 355.7 | 14.15 | unknown * |
| 3 | 0.73 | 0.4 | 0.75 | 19.6 | 10.11 | 0.77 | 7.4 | 207.9 | 8.27 | unknown * |
| 4 | 0.86 | 9.0 | 0.89 | 17.5 | 9.03 | 0.92 | 5.4 | 483.3 | 19.23 | unknown * |

Track 1, ID:



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|--------|--------|--------------------|
| 1 | 0.08 | 2.5 | 0.10 | 122.1 | 67.51 | 0.13 | 0.1 | 1103.7 | 47.11 | unknown * |
| 2 | 0.55 | 6.2 | 0.55 | 14.4 | 7.97 | 0.58 | 5.8 | 202.9 | 8.66 | unknown * |
| 3 | 0.66 | 9.1 | 0.70 | 23.3 | 12.86 | 0.71 | 14.6 | 470.6 | 20.09 | unknown * |
| 4 | 0.76 | 12.0 | 0.77 | 21.1 | 11.66 | 0.82 | 4.0 | 565.9 | 24.15 | unknown * |

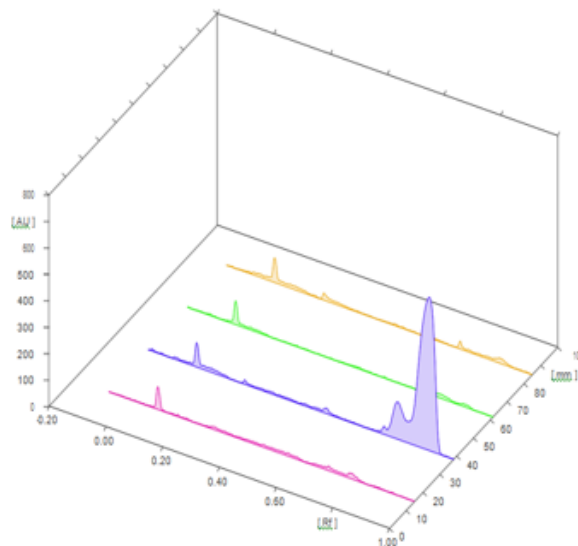
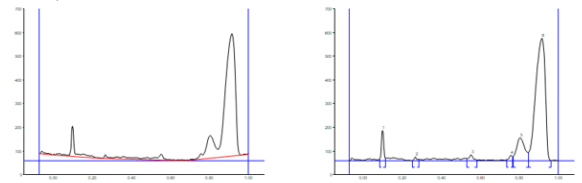


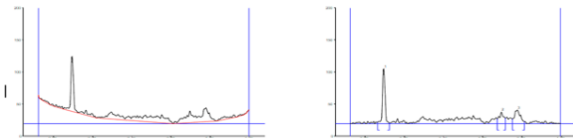
Fig. 6: All tracks at wavelength 300nm

Track 2, ID:



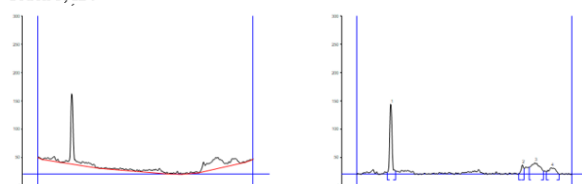
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|---------|--------|--------------------|
| 1 | 0.08 | 4.8 | 0.09 | 127.2 | 15.86 | 0.11 | 10.1 | 1154.4 | 4.80 | unknown * |
| 2 | 0.25 | 0.0 | 0.26 | 15.6 | 1.95 | 0.28 | 3.8 | 150.7 | 0.63 | unknown * |
| 3 | 0.53 | 7.8 | 0.55 | 24.5 | 3.05 | 0.58 | 2.3 | 410.5 | 1.71 | unknown * |
| 4 | 0.73 | 3.5 | 0.75 | 22.6 | 2.82 | 0.76 | 15.9 | 301.7 | 1.26 | unknown * |
| 5 | 0.77 | 16.6 | 0.80 | 95.9 | 11.96 | 0.84 | 33.5 | 3051.9 | 12.70 | unknown * |
| 6 | 0.85 | 33.9 | 0.91 | 516.2 | 64.37 | 0.96 | 0.0 | 18968.4 | 78.91 | unknown * |

Track 1, ID:



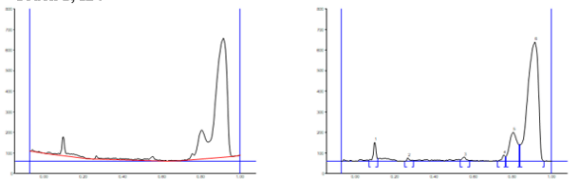
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|-------|--------|--------------------|
| 1 | 0.06 | 2.3 | 0.10 | 85.3 | 68.44 | 0.12 | 0.9 | 869.6 | 51.45 | unknown * |
| 2 | 0.68 | 7.4 | 0.70 | 18.0 | 14.46 | 0.72 | 8.6 | 320.9 | 18.99 | unknown * |
| 3 | 0.75 | 7.2 | 0.78 | 21.3 | 17.11 | 0.82 | 3.3 | 499.6 | 29.56 | unknown * |

Track 3, ID:



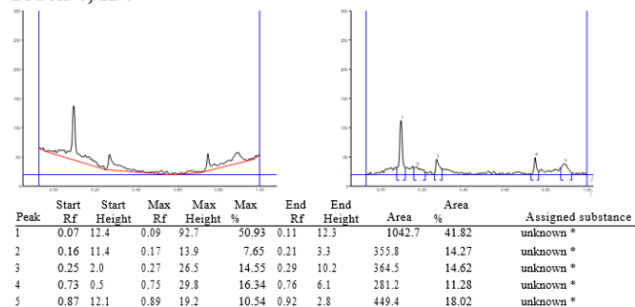
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|--------|--------|--------------------|
| 1 | 0.08 | 3.6 | 0.09 | 124.3 | 71.69 | 0.12 | 5.5 | 1132.0 | 48.65 | unknown * |
| 2 | 0.73 | 1.5 | 0.75 | 16.9 | 9.74 | 0.76 | 10.3 | 165.5 | 7.11 | unknown * |
| 3 | 0.78 | 12.8 | 0.81 | 20.5 | 11.80 | 0.86 | 5.7 | 717.2 | 30.82 | unknown * |
| 4 | 0.87 | 4.4 | 0.90 | 11.8 | 6.78 | 0.93 | 0.7 | 312.1 | 13.41 | unknown * |

Track 2, ID:



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|---------|--------|--------------------|
| 1 | 0.06 | 4.6 | 0.09 | 92.0 | 10.48 | 0.11 | 12.4 | 986.4 | 3.29 | unknown * |
| 2 | 0.25 | 0.2 | 0.26 | 16.7 | 1.90 | 0.29 | 3.7 | 195.2 | 0.65 | unknown * |
| 3 | 0.53 | 6.3 | 0.55 | 20.0 | 2.28 | 0.58 | 3.4 | 345.0 | 1.15 | unknown * |
| 4 | 0.72 | 0.8 | 0.76 | 30.2 | 3.44 | 0.76 | 21.4 | 389.1 | 1.30 | unknown * |
| 5 | 0.77 | 22.0 | 0.80 | 140.6 | 16.01 | 0.84 | 80.1 | 4349.8 | 14.52 | unknown * |
| 6 | 0.84 | 80.3 | 0.91 | 578.7 | 65.89 | 0.96 | 0.4 | 23701.2 | 79.09 | unknown * |

Track 4, ID:



Track 3, ID:

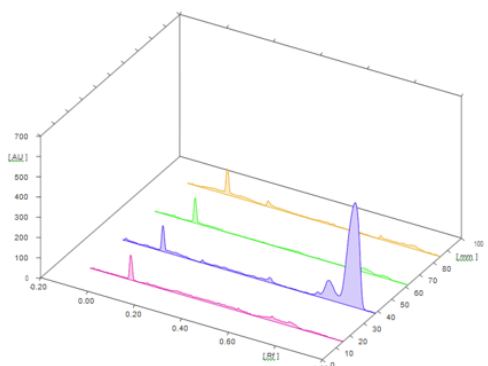
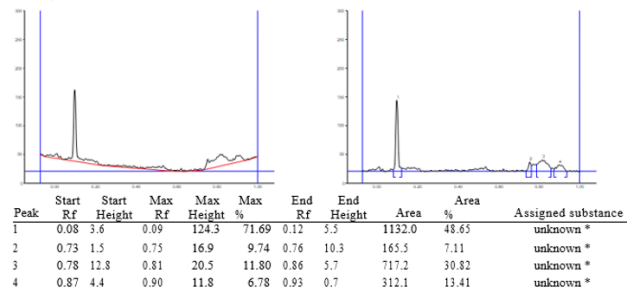
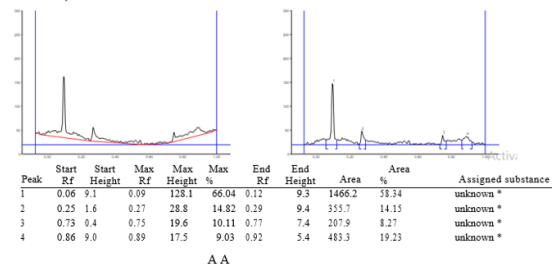


Fig. 7: All tracks at wavelength at 350 nm

Track 4, ID:



Track 1, ID:

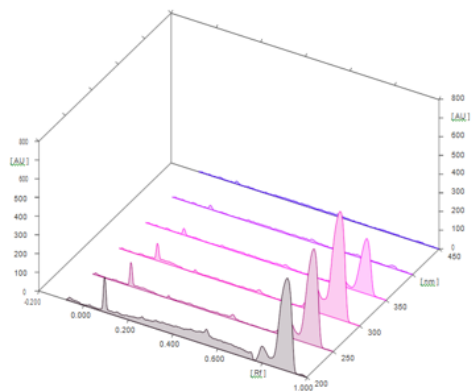
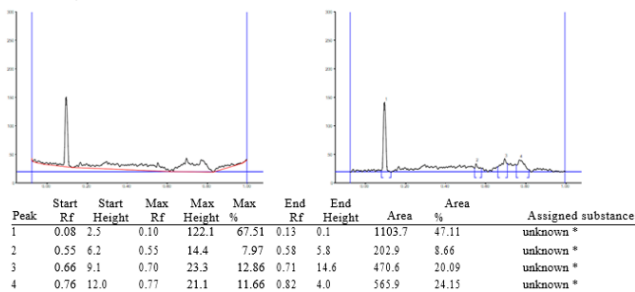
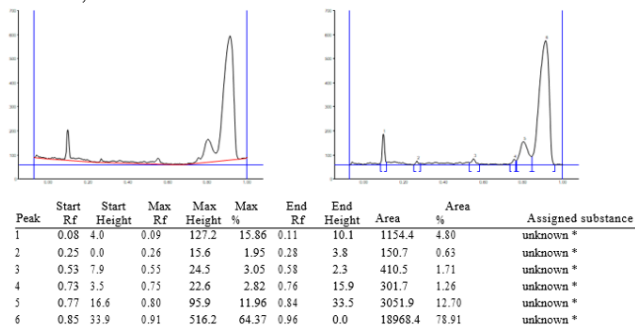
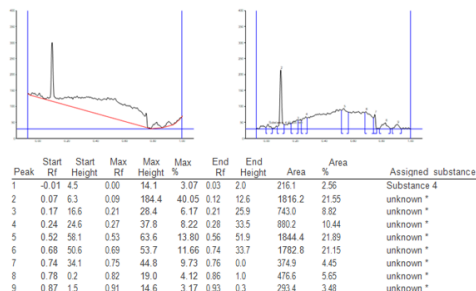


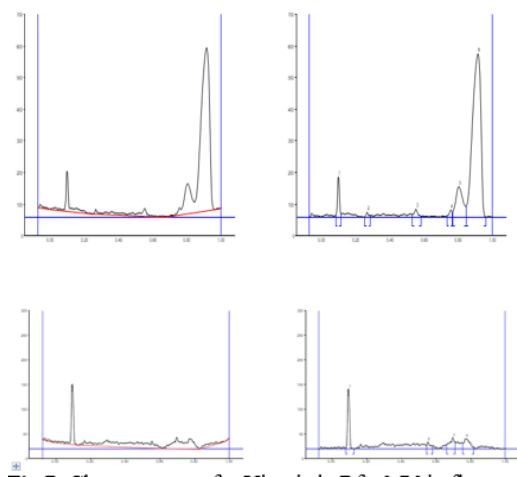
Fig. 8: All tracks at Wavelength 250nm

Track 2, ID:



Track 3, ID:





For vincristine, simultaneous estimation of vincristine was performed by HPTLC on a silica gel plate using toluene-methanol-diethylamine (8.75: 0.75: 0.5, v/v/v) as the mobile phase. The method was validated as per the ICH guidelines. The R_f value was found to be 0.76 for flower and 0.80 for leaves at 250nm which shows the presence of vincristin in *Catharanthus roseus*.

4. Conclusion

Phytochemical screening and HPTLC method was developed for the determination of different types of alkaloids and vincristine respectively. The method was found to be simple, effective, rapid, specific and accurate for the analysis of alkaloids and vincristine in crude drug and can be adopted by any laboratory for formulation, identification and quality control of crude drugs.

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6. Source of Funding

None.

7. Conflict of Interest

None.

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