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APPLICATIONS OF CHROMATOGRAPHY FOR THE QUALITY EVALUATION OF HERBAL DRUGS AND FORMULATIONS

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Keywords:

Chromatography, HPTLC, Quality Control, Fingerprinting, Phytochemical Analysis, Standardization

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ABSTRACT

Chromatography is a powerful analytical technique widely used for the separation, identification, and quantification of chemical constituents in complex mixtures. In herbal drug research and quality control, chromatographic methods play a crucial role in ensuring the authenticity, purity, safety, and efficacy of medicinal plants and herbal formulations. This review highlights the applications of High-Performance Thin Layer Chromatography (HPTLC) for the quality control and standardization of some important crude drugs. The review discusses chromatographic fingerprinting and quantitative estimation of bioactive markers in medicinal plants such as *Piper longum, Glycyrrhiza glabra, Withania somnifera, Ocimum sanctum, Azadirachta indica, Andrographis paniculata, Tinospora cordifolia,Swertia chirata,Mesua ferrea, and Cucurbita pepo*. These applications demonstrate the significance of chromatography in standardization, detection of adulteration, quality assurance, and regulatory compliance of herbal medicines. Overall, chromatographic techniques, especially HPTLC, serve as indispensable tools for the comprehensive evaluation and quality control of herbal drugs and formulations.

Introduction

Chromatographic Conditions for HPTLC Analysis of *Piper longum*Fruit.

Piper longum is a spicy herb which belongs to family Piperaceae. Fruits are used as aromatic, stomachic, stimulants and carminative, stimulates taste buds and increases gastric juices.It is used to enhance bioavailability of certain drugs. The main phytochemical constituent present in the drug is Piperine.

Chromatographic Analysis-

1.Sample Preparation: Take 0.5g of sample material and add 10mL methanol and macerate it for 12h and filter.

2.Phytochemical referencesstandard (PRS): Dissolve a quantity of Piperine in methanol to produce a solution containing 1mg per ml.

3.Stationary phase: TLC aluminium plate precoated with silica gel 60F254.

4.Application: Apply sample separately to the plate with bands of 2mm, 10µL of each test solution, phytochemical references standard, and test solution, leaving 6mm between the bands.

5.Mobile phase: Toluene:chloroform:ethyl acetate (4:3:3), 20mL.

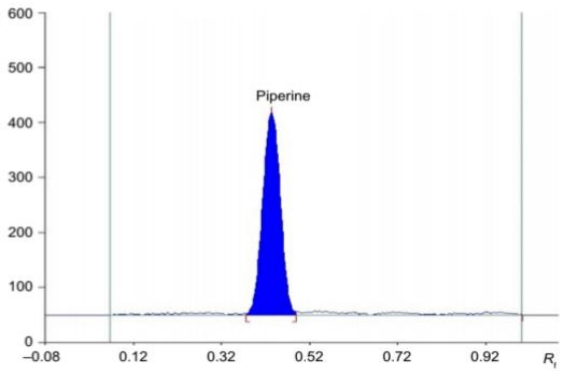
6.Chamber for development: Twin-trough chamber, 10cm×20cm.

7.Conditions: Saturate the chamber with a mobile phase for 30min. The plate should be developed vertically for 8cm.

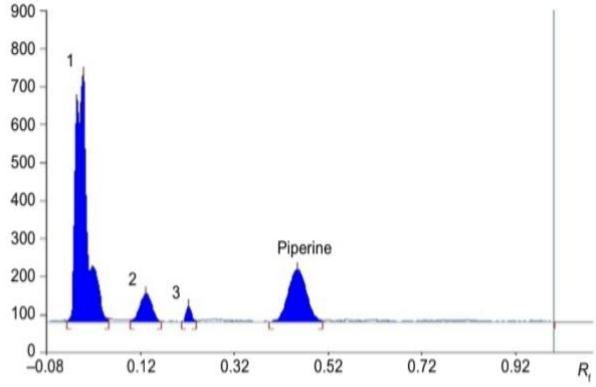
8.Detection: Examine in UV light at 254nm.

9.Amount of PRS: 2.64% (w/w).600

10.Rf value: 0.80-0.85



(Chromatogram of standard piperine)



(Chromatogram of *Piper longum* extract)

Chromatographic Conditions for HPTLC Fingerprint Analysis of *Glycyrrhiza glabra* Root Extract.

Chromatographic Analysis-

Glycyrrhiza glabra, commonly known as Mulethi, liquorice, or yasti, is an herbaceous plant which belongs to the family Leguminosae.The dried unpeeled roots and stolons are used as an expectorant and demulcent. The main phytochemical constituent present in the drug is glycyrrhizin.

1. Sample Preparation: Take 1g of sample material and add 10mL methanol and macerate it for 12h and filter.

2. Phytochemical references standard (PRS): Dissolve a quantity of glycyrrhizin in methanol to produce a solution containing 1mg per ml.

3. Stationary phase: TLC aluminium plate pre-coated with silica gel 60F254.

4. Application: Apply sample separately to the plate with bands of 2mm, 10µL of each test solution, phytochemical references standard, and test solution, leaving 6mm between the bands.

5. Mobile phase: n-butanol: acetic acid: water (12:3:4), 20mL.

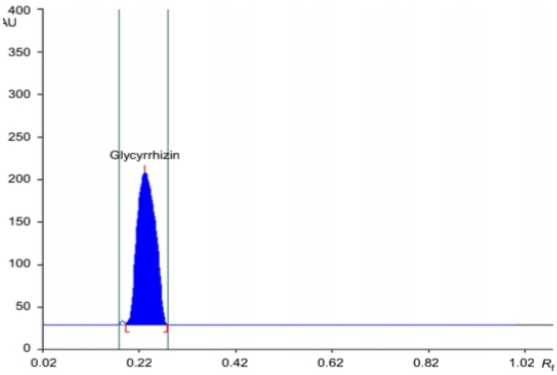
6. Chamber for development: Twin trough chamber, 10cm×20cm.

7. Conditions: Saturate the chamber with a mobile phase for 30min. The plate should be developed vertically for 8cm.

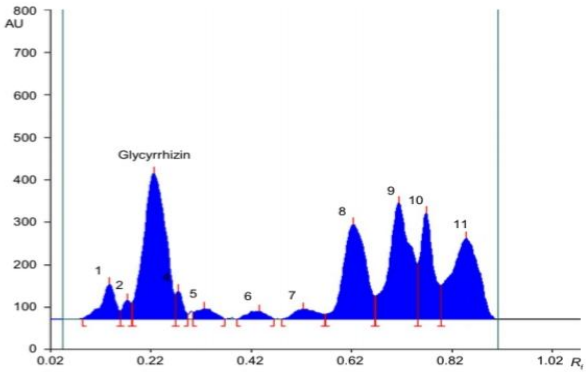
8. Detection: Examine in UV light at 254nm.

9. Amount of PRS: 1.78% (w/w).

10.Rf value: 0.30-0.38



(HPTLC chromatogram of glycyrrhizin)



(Chromatogram of *Glycyrrhiza glabra* extract)

Chromatographic Conditions for HPTLC Fingerprint Analysis of *Withania somnifera* Root Extract.

Chromatographic Analysis-

Withania somnifera, commonly known as Withania root or winter cherry, is a medicinal herb which belongs to the family *Solanaceae*. The dried roots and stem bases are used for their sedative and hypnotic effects, as an immunomodulatory agent, and for anti-stress activity; they also act as a respiratory stimulant with bradycardia and are utilized in the treatment of rheumatism and gout. The main phytochemical constituent present in the drug is withanolide**1. Sample Preparation:** Take 1g of sample material and add 10mL methanol and macerate it for 12hrs and filter.

2. Phytochemical references standard (PRS): Dissolve a quantity of withanolide A in methanol to produce a solution containing 1mg per ml.

3. Stationary phase: TLC aluminium plate pre-coated with silica gel 60F254.

4. Application: Apply sample separately to the plate with bands of 2mm, 10µL of each test solution, phytochemical references standard and test solution leaving 6mm between the bands.

5. Mobile Phase: Chloroform and methanol (9:1), 20mL.

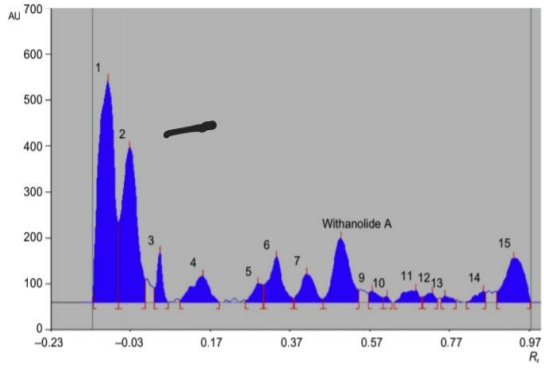
6. Chamber for development: Twin-trough chamber, 10cm×20cm.

7. Conditions: Saturate the chamber with a mobile phase for 30min. The plate should be developed vertically for 8cm.

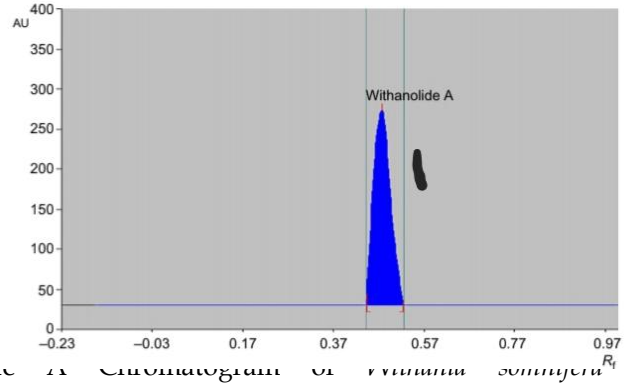
8. Detection: Examine in UV light at 254nm.

9. Amount of PRS: 0.6% (w/w).

10. Rf value: 0.55-0.75



HPTLC chromatogram of standard withanolide A



Chromatogram of *Withania somnifera*

Chromatographic Conditions for HPTLC Fingerprint Analysis of *Ocimum sanctum* Leaf Extract

Chromatographic Analysis-

Ocimum sanctum, commonly known as Holy basil, is a sacred herb which belongs to the family *Lamiaceae or Labiatae*. The fresh leaves are used as an aromatic, stimulant, spasmolytic, expectorant, and immunomodulator. The main phytochemicals present in the drug is eugenol.

1. Sample Preparation: Take 1g of sample material and add 10mL methanol and macerate it for 12h and filter.

2. Phytochemical references standard (PRS): Dissolve a quantity of eugenol in methanol to produce a solution containing 1mg per ml.

3. Stationary phase: TLC aluminium plate pre-coated with silica gel 60F254.

4. Application: Apply sample separately to the plate with bands of 2mm, 10µL of each test 0.5

5. Mobile Phase: Toluene: ethyl acetate: formic acid (90:10:01), 20mL

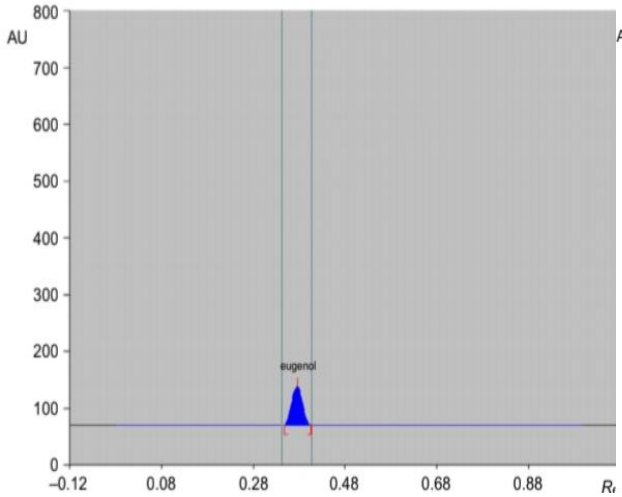
6. Chamber for development: Twin-trough chamber, 10cm×20cm.

7. Conditions: Saturate the chamber with a mobile phase for 30min. The plate should be developed vertically for 8cm.

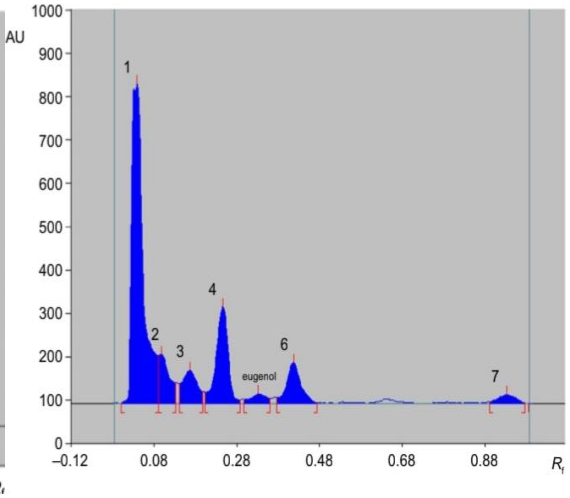
8. Detection: Examine in UV light at 254nm.

9. Amount of PRS: 0.13% (w/w).

10. Rf value: 0.50-0.61.



(Chromatogram of Eugenol)



(Chromatogram of *Ocimum sanctum* extract)

Chromatographic Conditions for HPTLC Fingerprint Analysis of *Azadirachta indica* (Neem) Leaf Extract

Chromatographic Analysis-

Azadirachta indica, commonly known as Neem or Indian Lilac, is a versatile tree which belongs to the family *Meliaceae*. The fresh leaves are used for their antibacterial, antifungal, antiviral, anti-inflammatory, antipyretic, and insecticidal properties; they are also widely utilized in skin treatments, dental care, and as an immunomodulator. The main phytochemical constituent present in the drug is Azadirachtin.

1. Sample Preparation: Take 1g of sample material and add 10mL methanol.

Macerate for 12 hours and filter.

2. Phytochemical References Standard (PRS): Dissolve a quantity of azadirachtin in methanol to produce a solution containing 1mg per ml.

3. Stationary Phase: TLC aluminium plate pre-coated with silica gel 60F254.

4. Application: Apply sample separately to the plate with band.

o Use 10µL of each test solution, phytochemical references standard, and test solution,

leaving 6mm between the bands.

5. Mobile Phase: Toluene: ethyl acetate: formic acid (5:4:1), 20mL.

6. Chamber for Development: Twin-trough chamber, 10cm × 20cm.

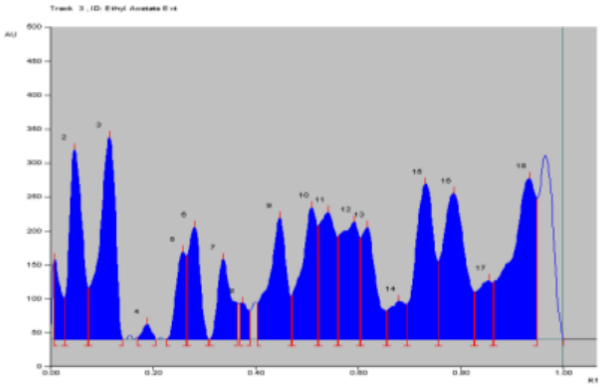
7. Conditions: Saturate the chamber with the mobile phase for 30 minutes.

Develop the plate vertically for 8cm.

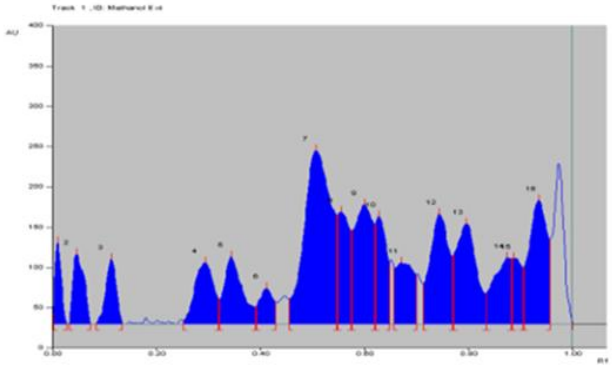
8. Detection: Examine under UV light at 254nm.

9. Amount of PRS: To be determined based on the specific sample analysis.

10. Rf value: 0.55-0.75



Chomatogram of ethyl acetate of *A.indica*



Chromatogram of methanol extract of *A.indica*

Chromatographic Conditions for HPTLC Analysis of *Kalmegh (Andrographis paniculata)*

Chromatographic Analysis-

Andrographis paniculata, commonly known as Kalmegh or Green Chiretta, is a bitter herb which belongs to the family *Acanthaceae*. The dried aerial parts are used for their hepatoprotective, anti-inflammatory, anti-microbial, and immune-modulatory properties. The main phytochemical constituent present in the drug is andrographolide.

1.Sample Preparation: Take 0.5g of Kalmegh sample material and add 10mL methanol.Macerate for 12 hours and filter.

2. Phytochemical Reference Standard (PRS): Dissolve a quantity of andrographolide in methanol to produce a solutioncontaining 1mg/mL.

4. Stationary Phase:TLC aluminium plate pre-coated with silica gel 60F254.

5. Application: Apply the sample and PRS separately to the plate with bands of 2mm.

Use 10µL of each test solution, leaving 6mm between the bands.

6. Mobile Phase: Toluene: Chloroform: Ethyl acetate (4:3:3), 20mL.

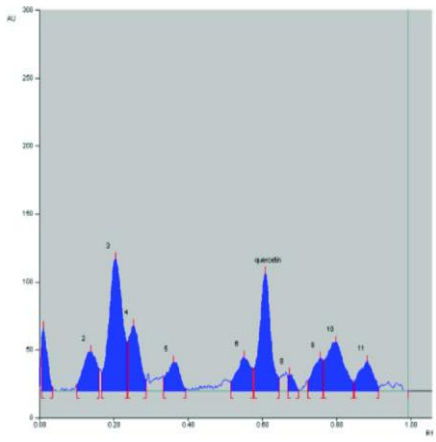
7. Chamber for Development: Twin-trough chamber, 10cm × 20cm.

Saturate the chamber with the mobile phase for 30 minutes.

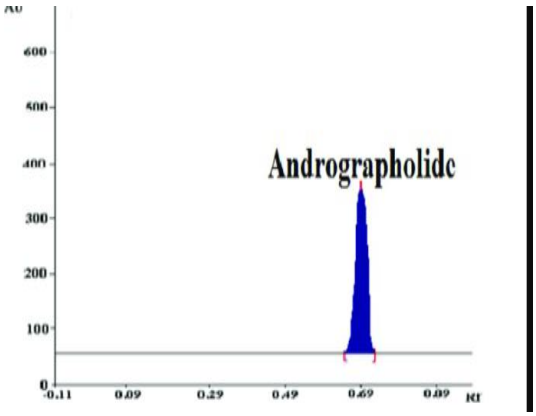
8. Development Conditions: Develop the plate vertically for 8cm.

9. Detection: Examine under UV light at 254nm.

10. Rf value: 0.55-0.75



Chomatogram of *Andrographis paniculata*



HPTLC chromatogram of Andrographolide

Chromatographic Conditions for HPTLC Fingerprint Analysis of *Tinospora cordifolia (Guduchi)* Stem Extract.

Chromatographic Analysis-

Tinospora cordifolia, commonly known as Guduchi or Giloy, is a climbing shrub which belongs to the family *Menispermaceae.* The dried stems are used for their immunomodulatory, antipyretic, anti-inflammatory, anti-diabetic, hepatoprotective, and antioxidant properties, making it highly effective in treating fever, managing diabetes, and improving immunity. The main phytochemical constituent present in the drug is berberine.

1. Sample Preparation: Take 1g of sample material and add 10mL methanol.

Macerate for 12 hours and filter.

2. Phytochemical References Standard (PRS): Dissolve a quantity of tinosporin in methanol to produce a solutioncontaining 1mg per milliliter.

3. Stationary Phase:TLC aluminum plate pre-coated with silica gel 60F254.

4. Application: Apply sample separately to the plate with bands of 2mm.

Use 10µL of each test solution, phytochemical references standard, and test
solution, leaving 6mm between the bands.

5. Mobile Phase: Toluene: ethyl acetate: formic acid (5:4:1), 20mL.

6. Chamber for Development: Twin-trough chamber, 10cm × 20cm.

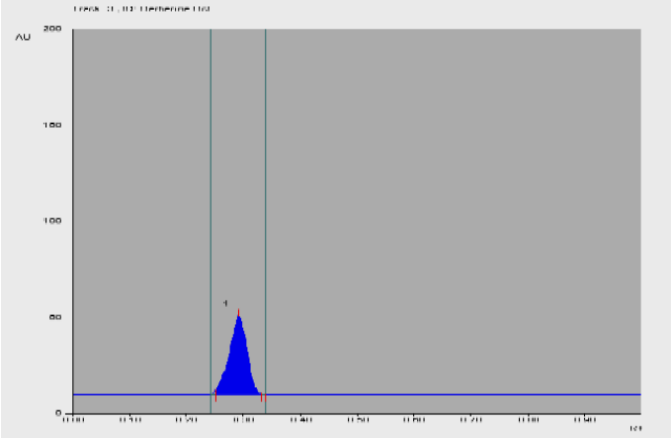
7. Conditions: Saturate the chamber with the mobile phase for 30 minutes.

Develop the plate vertically for 8cm.

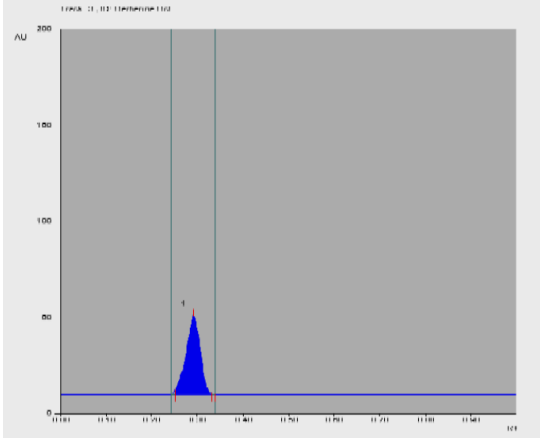
8. Detection: Examine under UV light at 254nm.

9. Amount of PRS: To be determined based on the specific sample analysis.

10. Rf value: 0.58



HPTLC chromatogramof *Tinospora cordiofolia*



HPTLC Chromatogram of berberine

Chromatographic conditions for HPTLC Fingerprint Analysis of *Swertia chirata* (Chirayita) Leaf extract

Chromatographic Analysis-

Swertia chirata species, commonly known as *Agathotes chirayita* or chiretta, is a bitter medicinal herb which belongs to the family Gentianaceae. The dried plants are used in the management of malaria, diabetes, and liver disorders, and are valued for their potent anti-oxidant properties. The main phytochemical constituent present in the drug is ursolic acid.

1. Sample Preparation: Take 1g of sample material and add 10mL methanol and macerate it for 12h andfilter

2. Phytochemical references standard (PRS): Dissolve a quantity of ursolic acid in methanol to produce a solution containing 1mg perml

3. Stationary phase: TLC aluminium plate pre coated with silica gel 60F254

4.Application: Apply sample separately to the plate with bands of 2mm,10µL of each test solution, phytochemical references standard, and test solution, leaving 6mm between the bands.

5. Mobile phase: Toluene: ethyl acetate: formic acid (7:3:0.2),20mL

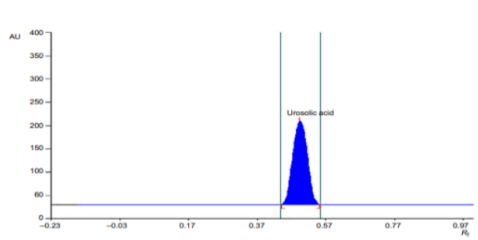
6. Chamber for development: Twin-trough chamber, 10cm×20cm

7. Conditions: Saturate the chamber with mobile phase for 30min. The plate should be developed vertically for 8cm.

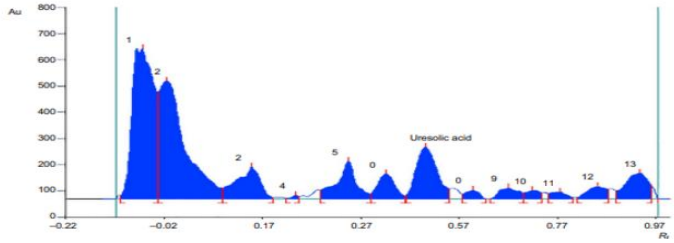
8. Detection: Examine in UV light at 254nm.

9. Amount of PRS: 2.68% (w/w).

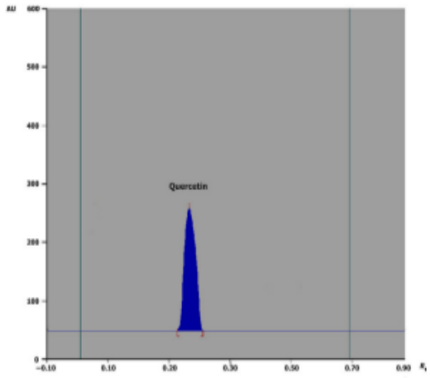
10. Rf value: 0.42-0.48



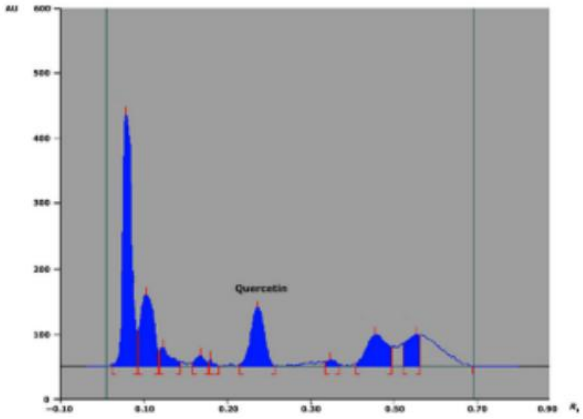
HPTLC chromatogram of Ursolic acid



HPTLC Chromatogram of *Swertia chirata*



HPTLC Chromatogram of Quercetin



Chromatogram of *Messua ferra* extract

Chromatographic Conditions for HPTLC Fingerprint Analysis of *Messua ferra* Root Extract

Chromatographic Analysis-

Mesua ferrea, commonly known as Ceylon ironwood or cobra saffron, is a therapeutic tree which belongs to the family *Calophyllaceae*. Various parts of the tree, including the flowers, leaves, bark, and seeds, are used for their antioxidant, antimicrobial, analgesic, blood purifying activities. Main phytochemical constituent present in the drug is Quercetin.

1.Sample Preparation: Take 1g of sample material and add 10mL methanol and
macerate it for 12h and filter

2.Phytochemical references standard (PRS): Dissolve a quantity of Quercetin in methanol to produce a solution containing 1mg per ml.

3.Stationary phase: TLC aluminum plate precoated with silica gel 60F254.

4.Application: Apply sample separately to the plate with bands of 2mm, 10µL of each test solution,phytochemical references standard, and test solution, leaving 6mm between the bands

5.Mobile Phase:n-Butanol:acetic acid:water (12:3:4),20mL

6.Chamber for development: Twin-trough chamber, 10cm×20cm

7.Conditions: Saturate the chamber with mobile phase for 30min. The plate should be developed vertically for 8cm.

9.Amount of PRS:2.10% (w/w)

10.Rf value: 0.55-0.77

Chromatographic Conditions for HPTLC Fingerprint Analysis of *Cucurbita pepo* Fruit Extract

Chromatographic Analysis-

Cucurbita pepo, commonly known as Autumn pumpkin, pumpkin, or pumpkin vine, is a trailing plant which belongs to the family *Cucurbitaceae*. The dried and ripe seeds are used to increase urination, help relieve bladder and prostate discomfort, and reduce swelling in the prostate. The main phytochemical constituent present in the drug is cucurbitacin E.

1.Sample preparation: Take 2g of sample material and add 20mL methanol and
macerate it for 6h and filter

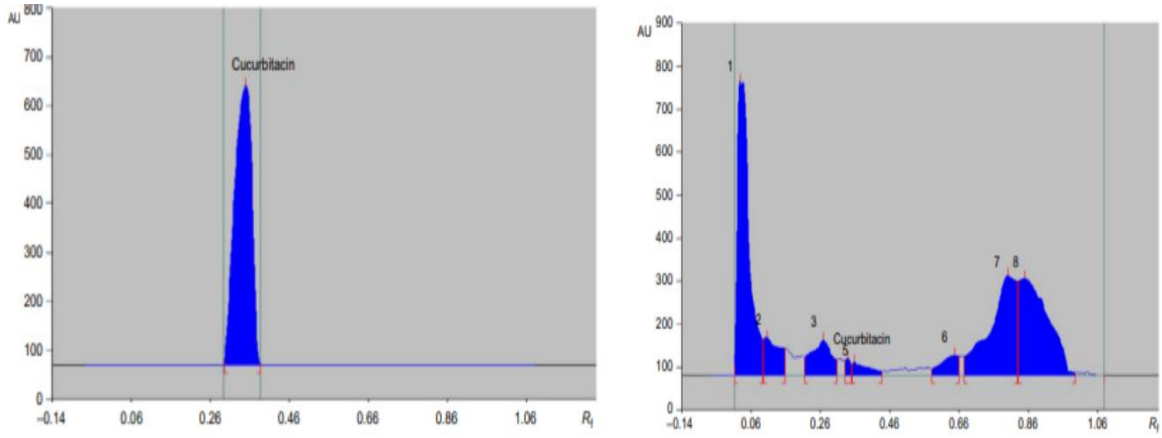
2.Phytochemical references standard (PRS): Dissolve a quantity of Cucurbitacin E in methanol to produce a solution containing 1mg per ml

3. Stationary phase: TLC aluminum plate precoated with silica gel 60F254

4. Application: Apply sample separately to the plate with bands of 2mm, 10µL of each test solution,phytochemical references standard, and test solution, leaving 6mm between the bands

5.Mobile phase: Petroleum ether:ethyl acetate (7:3), 20mL

6. Chamber for development: Twin-trough chamber, 10cm×20cm
7. Conditions: Saturate the chamber with mobile phase for 30min. The plate should be developed vertically for 8cm
8. Detection: Examine in UV light at 254nm
9. Amount of PRS- 0.18% (w/w)
10.Rf value: 0.75-0.88



Typical HPTLC chromatogram of standard Typical chromatogram of Cucurbita cucurbitacin

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