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Thin Layer Chromatography Analysis of *Scoparia Dulcis* Linn (Scrophulariaceae)

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Abstract

Herbal medications are crucial for treating liver ailments. In India, numerous medicinal plants and their blends are extensively employed for this purpose. There's a prevalent chromatography method for chemical compound separation. It employs a stationary phase, a thin layer of adsorbent material affixed to an inert sheet, for this purpose.

Keywords- Herbal drugs, Chromatography, Thin layer chromatography.

1 INTRODUCTION

Herbal remedies are vital in treating liver conditions, and in India, various medicinal plants and their combinations are commonly utilized for this purpose. Besides the known medicinal plants, there are numerous unexplored potential candidates that warrant further study for their effectiveness against liver disorders. Scoparia dulcis linn, a smooth under-shrub with small white flowers, is abundant in wastelands and fallow fields. This plant has been extensively used in traditional medicine. The infusion of its leaves is employed for fever, cough, and bronchitis, and as a gargle for toothaches. Additionally, the plant's decoction is utilized for kidney issues, while its leaf juice, taken with yogurt, is used to treat jaundice. Amellin-an, an antidiabetic compound found in its leaves, exhibits usefulness in addressing anemia, albuminuria, ketonuria, retinitis, and other diabetic complications. [1-11].

1.1 Thin Layer Chromatography (TLC)

This chromatography technique is extensively utilized for chemical compound separation. It employs a stationary phase, a thin layer of adsorbent material affixed to an inert sheet. A liquid phase containing the solution to be separated, dissolved in a suitable solvent, is pulled through the plate via capillary action, thereby separating the components of the solution. Its applications are diverse, ranging from determining plant pigments to detecting pesticides or insecticides in food, analyzing dye compositions of fibers in forensics, and identifying compounds within substances. It's a rapid and effective method for monitoring organic reactions and also finds utility in monitoring column chromatography.

2 MODE OF SEPARATION

In TLC, separations occur by distributing a mixture of substances between a stationary phase and a mobile phase. The mobile phase, a developing liquid, moves up the stationary phase, carrying the samples. The separation of sample components on the stationary phase depends on their adsorption onto the stationary phase compared to their dissolution in the mobile phase.

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2.1 Types of plates

1) Based on mode of chromatography

- Normal phase: Adsorbent material polar and mobile phase non polar, mainly plate with code $F_{254+366.}$
- Reverse phase: Adsorbent material non-polar and mobile phase polar mainly plate with code RP, RPF_{254S} (means reverse phase plate with blue fluorescence at 254 nm).

2) Based on type of use

- Analytical plate(100-250 µm)
- Preparative plate(1-2 mm)

3) Based on modification of plates

- Amino(NH_2) Plates
- Cyano(CN) Plates
- Chiral layer(CHIR) Plates
- Hydrophilic layer(DIOL) Plates
- Impreginated Plates

4) Based on mode of preparation

- Manually coated plates
- Precoated plates(20x20cm)

2.2 Type of stationary phase

- Cellulose: Fibrous cellulose, cellulose with starch as binder, microcrystalline cellulose, microcrystalline cellulose with fluorescent indicator.
- Silica: Silica gel G (Gypsum; $CaSO_4.H_2O$), silica gel GF (fluorescent), silica gel with starch.

2.3 Type of mobile phase

Pentane, hexane, heptane, cyclohexane, toluene, xylene, benzene, diethyl ether, dichloromethane, n-butanol, chloroform, ethyl acetate, dioxane, acetone, methanol, ethanol, acetonitrile, acetic acid, water etc.

3 STEPS INVOLVED IN TLC

3.1 Coating of plate

TLC plates are created by blending an adsorbent, like silica gel, with a small quantity of an inert binder such as calcium sulfate (gypsum) and water. This blend is applied as a thick slurry onto an inert carrier sheet, typically glass, thick aluminum foil, or plastic, and the resulting plate is then dried. For analytical use, the adsorbent layer is usually around 0.1–0.25 mm thick, while for preparative TLC, it's typically 1–2 mm thick.

3.2 Activation of Plate

The coated plate is left to dry naturally and then activated. Activation of the TLC plate involves heating it in an oven for thirty minutes at 110°C.

3.3 Application of sample

The sample should be applied using a clean capillary. The application volume should range between 1-10 µl, and it's important to avoid applying high concentrations to prevent tailing effects.

a) Thin circular spot

b) Band form

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3.4 Chamber

The chamber's shape and size are determined by the plate size. Various types of chambers like rectangular glass chambers, twin trough chambers, V-shaped chambers, and circular chambers are commonly utilized. The condition of the chamber significantly affects the separation outcome, hence ensuring proper saturation is crucial for optimal separation. Inadequate saturation can lead to edge effects and spot tailing, along with increased solvent consumption in unsaturated chambers.

3.5 Development and Drying

Ascending, descending, two-dimensional, horizontal, multiple over-run, circular, multi-dimensional, etc., are the most common methods of chromatographic development. When the solvent front reaches 75% of the plate length, it should be removed and dried thoroughly.

3.6 Detection and visualization

- For colored spots- visual technique
- UV active spots at wavelength 254 & 366 nm.
- Non UV active Derivatization reaction by help of various spray reagents.

4 EVALUATION OF TLC

TLC is applicable for both qualitative and quantitative analysis, with HPTLC used for quantitative purposes. The Rf value (retention factor) is commonly employed as a parameter for qualitative assessment.

Rf = Distance traveled by spot / distance traveled by solvent front

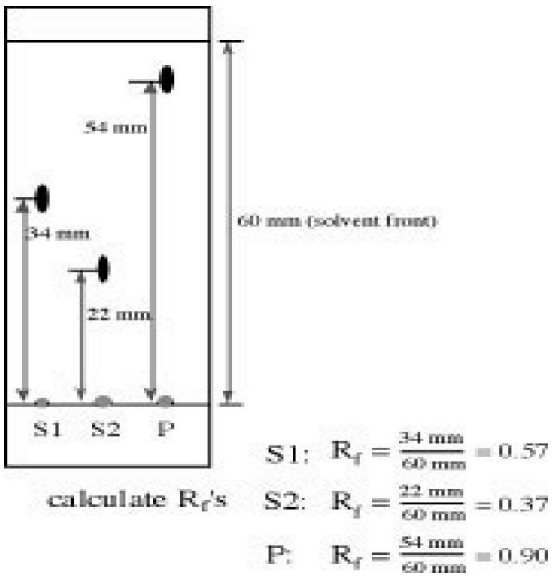

Figure 1: Measurement of distance for calculation of Rf values in a developed TLC plate

5 MODIFICATION OF TLC- HPTLC

High performance thin layer chromatography (HPTLC) is an advanced technique compared to TLC, operating on the same principle. However, HPTLC offers automated sample application with known sample volumes, automated scanning, Rf calculation, and densitometry. Additionally, quantitative analysis is achievable with HPTLC.

5.1 Uses of TLC

- To determine the number of components in a mixture.
- To determine the identity of two substances.

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- To monitor the progress of a reaction.
- To determine the effectiveness of a purification.
- To determine the appropriate conditions for a column chromatographic separation.
- To monitor column chromatography.

6 CONCLUSION

In this study, the PDM extract from Scoparia dulcis L. was discovered to contain terpenoids and phytosterols. The TLC analysis of the extract revealed the presence of 11 compounds. Furthermore, the extract exhibited dose-dependent in vitro antioxidant activity against DPPH free radicals and in vivo hepatoprotective activity against liver damage induced by CCl4. These observed effects of the extract are likely due to its terpenoid content.

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