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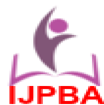

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A Review: Thin Layer Chromatography of Plant Pigments

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Abstract

Bio-autography on thin-layer chromatographic (TLC) plates is a means of detecting the biological activity of a sample which has migrated on the plate with a suitable solvent. It only requires small amounts of sample and is ideal for the investigation of plant constituents, which often occur as complex mixtures. It can be used for the target-directed isolation of these constituents. In contrast to HPLC, many samples can be run at the same time on TLC. Organic solvents, which cause inactivation of enzymes or death of living organisms, can be completely removed before biological detection. Many bioassays are compatible with TLC. Antimicrobial, radical scavenging, antioxidant activities and enzyme inhibition feature among the tests that are employed.

Keywords: Thin-layer chromatography, Detectionmethods, Bio-autography Bioactivity Plants Phytochemicals

Introduction

Thin layer chromatography can be used to: Monitor the progress of a reaction, identify compounds present in a given substance, determine the purity of a substance. Separation of compounds is based on the competition of the solute and the mobile phase for binding places onthe stationary phase. For instance, if normal phase silica gel is used as the stationary phase it can be considered polar. Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places.

Principle of TLC 1: Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind 1.

R*f* values: The behavior of an individual compound in TLC is characterized by a quantity Known as R*f* and is expressed as a decimal fraction.

R*f* = Distance of center of spot from starting point/ Distance of solvent front from starting point

The R*f* value is a constant for each component only under identical experimental condition. It depends upon number of factors as;

Nature of adsorbent: Different adsorbents will give different R*f* value for same solvent. Reproducibility is only possible for given adsorbent of constant particle size and binder.

The mobile phase: The purity of solvents and quantity of solvent mixed should be strictly controlled. It should be made freshly for each run if one of the solvents is very volatile or hygroscopic.

Temperature: As the temperature is increased, Volatile solvents evaporate more quickly,

solvents run faster, and R*f* values generally decrease slightly.

Thickness of layer: Standard plates approximately 250 micrometer is the preferable thickness of layer. Below 200, the R*f* values vary considerably.

Developing tank: It is important that saturated conditions are attained for running TLC plates.This is best accomplished by using small tanks with filter paper liners and sufficient solvent, and by leaving the tank to equilibrate for at least 30 minutes before running the plates.

Mass of sample: Increasing the mass of sample on the plate will often increase the R*f* of drug, especially if it normally tails in the system

Chromatographic Technique: Depending upon the development technique used i.e. ascending, descending, horizontal etc, the R*f* value change for the same solvent system.

Plate preparation: The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the adsorbent layer is typically around 0.1- 0.25 mm for analytical purposes and around 0.5- 2.0 mm for preparative TLC.

Capillary spotters: Place a melting point capillary and in the dark blue part of the Bunsen burner flame. Allow the capillary to cool down, and then break it in the middle. Make sure to break off the closed end on one of them.

Spotting the plate: The thin end of the spotter is placed in the dilute solution; the solution will rise up in the capillary (capillary forces).

Location of spots: The position of various solutes separated by TLC can be located by various methods. Colored substances can be seen directly when viewed against stationary phase, while colorless substances can be detected only by making them visible by making use of some spraying agent.

Development solvents: Solvent systems range from non-polar to polar solvents. Non-polar solvents are generally used, as highly polar solvents cause the adsorption of any component of the solvent mixture. Commonly used development solvents are petroleum ether, carbon tetrachloride, pyridine, glycol, glycerol, diethyl ether, formamide, methanol, ethanol, acetone, and n-propanol.

Mobile Phase: For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvents. As the mobile phase moves pass the surface of the silica gel it transports the analyte pass the particles of the stationary phase.

Visualization:

If not, they can sometimes be visualized by shining ultraviolet light on the plate or by allowing the plate to stand for a few minutes in a closed container in which the atmosphere is saturated with iodine vapor. Sometimes the spots can be visualized by spraying the plate with a reagent that will react with one or more of the components of the sample.

Plant Profile

Lemongrass (*Cymbopogon Citratus*)

Classification:

Family Poaceae : Grass family

Botanical Name: Cymbopogon citratus

Family:

Graminae

Hibiscus Rosa-Sinensis

Family: Malvaceae

Genus: Hibiscus

Aegle Marmelos

Family: Rutaceae

Genus: AegleCorrea

Materials and Methods

Materials:

Various Materials Used For Experiment:

Chemicals:

1.Ethanol	2.Methanol
3.Chloroform	4.Conc.H2SO4
5.Ferric chloride	6.NAOH
7.Conc. HCL	8.Distilled water
9.Mayerreagent	10.Wagner reagent

11.Silica Gel G	12.n-hexane
13.Formic acid	14.Ethyl acetate

Apparatus:

1.Ultra Violet apparatus
2.TLC Chamber
3.Hot Air Oven

Glasswares:

1.Beaker	2.Glassrod
3.Measuring cylinder	4.Test tube
5.Petridish	6.Test tube stand
7.Conical flask	8.TLC plates
9.Funnel	10.Boiling test tube

Methods:

Extraction Method:

Lemongrass *(Cymbopogon Citratus)*

A mass of 50 g dried powdered *Cymbopogon citratus* was suspended in 100 ml distilled water

↓

filtered using a filter paper to obtain water soluble

↓

water insoluble portions by maceration for 5 days at room temperature.

↓

The water insoluble fraction was partitioned with ethanol, chloroform, and acetone to give the ethanol, chloroform and acetone fractions respectively.

↓

The extract was concentrated using evaporation at reduced pressure. It was dried on an evaporating dish at a temperature of 50°C to 60°C to a semi-solid form.

↓

A gel semi-solid greenish substance was obtained for both samples.

↓

All the extracts were stored in a well corked universal bottle for further analysis.

Aegle marmelos

↓

The leaves of the plant were properly washed in tap water and rinsed in distilled water.

↓

The rinsed leaves were hot air-dried for 3 days.

The dried leaves of each plant were pulverized using pestle mortar to obtain a powdered form which was stored in airtight glass containers at 4°C until used.

↓

10 g of powdered sample was soaked in distilled water and methanol (200 mL and 100 mL) separately for 12 hrs at room temperature.

↓

The extracts were then filtered and concentrated to a final volume of 50 mL and subjected to phytochemical analysis.

Percent Yield Definition and Formula: Percent yield is the percent ratio of actual yield to the theoretical yield. It is calculated to be the experimental yield divided by theoretical yield multiplied by 100%.

Percent Yield Formula:

The equation for percent yield is:

percent yield = (actual yield/theoretical yield) x 100%

Phytochemical Screening

Phytochemicals	Tests	Reagents
Alkaloids	Dragendorff's test (Raaman, 2006)	Dragendorff reagent
	Wagner's test (Raaman, 2006)	Wagner reagent
Flavonoids	Ammonia test (Rahul *et al.*, 2010)	1% NH3
	Sodium hydroxide test (Ajayi *et al.*, 2011)	20% NaOH, HCl
Tannins	Ferric chloride test (Raaman, 2006)	5% FeCl3
	Gelatin test (Rahul *et al.*, 2010)	1% gelatin solution containing 10% NaCl
	Lead acetate test (Raaman, 2006)	10% lead acetate
Saponins	Foam test (Ajayi *et al.*, 2011)	20ml distilled water (mixed vigorously for 15 min)
Terpenoids	Salkowski test (Ajayi *et al.*, 2011)	0.5ml chloroform, 1ml conc. H2SO4

Tlc Methods:

Thin Layered Chromatography was carried out by using the solvent system chloroform, n-hexane , ethyl acetate and n-hexane and ethyl acetate are mixed in the ratio 6:4:4 and 9:1 respectively. Total volume of solvent system prepared was 14 ml that was kept undisturbed for 20 minutes in TLC chamber for saturation to take place. Extract dilutions (1mg/ml) was prepared. Spotting of both the extracts was done and TLC plate was kept in theTLC chamber to obtain bands that were visualized under UV chamber at 246 and 366 nm.

Result and Conclusion

Thin Layer Chromatography:

$$Rf = \frac{\text{Distance of center of spot from starting point}}{\text{Distance of solvent front from starting point}}$$

Sample	Rf Value	Result
Lemongrass	2cm/11.2cm	0.1785cm
Hibiscus	2cm/10.4cm	0.1923cm
Aegle Marmelos	2cm/12cm	0.166cm

References:

1. Huner, N. P. A., Öquist, G., and Sarhan, F.

(1998) Energy balance to light and cold. Trends Plant Sci. 6, 224–230.

2. Young, A. J. (1993) Carotenoids in pigment-protein complexes, in Carotenoids in Photosynthesis (Young, A. and Britton, G., eds.), Chapman and Hall, New York, pp. 72–95.
3. Britton, G. (1993) Structure and Nomenclature, in Carotenoids in Photosynthesis (Young, A. and Britton, G., eds.), Chapman and Hall, New York, pp. 1–15.
4. Niyogi, K. K., Bjökman, O., and Grossman, A. (1997) The roles of specific xanthophylls in photoprotection. Proc. Natl. Acad. Sci. USA 94, 14,162–14,167.
5. Demmig-Adams, B. and Demmig-Adams, W. W. (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci.