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Thin Layer Chromatography Profiling of the Medicinal Plant Solanum Nigrum L. from the Local Area of the District Anantnag of Jammu and Kashmir

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Abstract: Medicinal plants contain different bioactive compounds which have great importance for the health of individuals and communities. These compounds produce definite physiological action on the human body. Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. Thin layer chromatography is a simple and efficient method used to identify and quantify secondary metabolites in herbal drugs. The present study was carried out to verify the thin layer chromatographic (TLC) profiling of Solanum nigrum L.using different solvents and it showed different R_F value.The results obtained in the present investigation indicated that the plant is rich source of secondary metabolite i.,e. alkaloids.

Key words: Medicinal Plant, TLC, Secondary Metabolite, R_F value, Drugs.

INTRODUCTION

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures into their components. All forms of chromatography work on the same principle. They all have a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mixture is dissolved in a fluid called the mobile phase which carries it through a structure holding another material called the stationary phase. The mobile phase flows through the stationary phase and carries the components of the mixture with it. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.There are different types of chromatography such as Column chromatography, paper chromatography etc. Among them thin layer chromatography (TLC) is a widely employed laboratory technique is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents.

MATERIALS AND METHODS

Thin layer chromatography (Trease and Evans, 1989; Kokate *et al.*, 2006)
The petroleum ether, chloroform and ethyl acetate extract obtained from the *Solanum nigrum* L. were subjected to purification process by chromatographic techniques. TLC was produced with the aim of identifying the individual substances in a mixture and also testing for purity or for separation of mixtures.

Thin layer chromatography (TLC) is a simple and inexpensive analytical technique that can quickly and efficiently separate quantities of less than ten micrograms of material. TLC has many applications in the organic laboratory. TLC is used for the rapid analysis of reagent and product purity, or to quickly determine the number of compounds in a mixture. Also, by comparing an unknown compound's behavior to the behaviors of known standard compounds, mixture compounds can be tentatively identified. TLC is performed on a sheath of glass, plastic or aluminum foil which is coated with a thin layer of adsorbent material, usually silica gel or cellulose. This layer of adsorbent is known as stationary phase. The plate or sheath is placed in a chamber containing a small amount of solvent which acts as mobile phase. The height of the solvent front and center of spots were measured in the form of Rf value. The Rf value indicates the position at which a substance was located in the chromatogram. A TLC experiment has three general stages: spotting, developing, and visualizing. In the present study TLC was done for crude extract, petroleum ether, and chloroform and ethyl acetate fractions to find out the probable number of compounds present in them.

Spotting a plate

The origin is marked, usually by drawing a thin line across the bottom of the plate with a pencil. The sample crude extract petroleum ether, chloroform and ethyl acetate fractions were dissolved in a volatile solvent such as ethanol. A glass capillary tube was used to apply a small amount of sample solution into the plate, keeping the sample in as small an area as possible. With practice, spots with diameters of 1-2 mm were produced.

Developing a plate

To develop the chromatogram, a piece of filter paper was placed along the walls of the developing chamber which contains a shallow layer of the appropriate eluent. The paper acts as a wick that adsorbs the eluent and ensures that, when the chamber was closed, its atmosphere was saturated with eluent vapour, minimizing evaporation from the plate. The spotted plates were placed into the chamber; the origin marked on the plate must be higher than the level of the eluent, to prevent the sample from dissolving from the plate into the eluent layer. When the eluent reached a point approximately 5 mm from top of the plate, the plates were removed from the chamber. The point that the eluent has reached is called the eluent front (or solvent front) and was immediately marked with a pencil.

Visualizing the compound

Upon development, a successful separation of coloured compounds will reveal distinct spots, indicating that the mixture compounds have separated. To make separated colorless compounds observable to the eye, the spots are treated in some way to make them visible. The process is called visualization. In the present study a number of developing solvent systems were tried during the study. The most informative and satisfactory resolution was taken as final solvent system. These solvent system were: Toluene: ethyl acetate: acetic acid (36:12:5), n-hexane:ethyl acetate:acetic acid (31:14:5), n-hexane:ethyl acetate:formic acid (31:14:5). TLC plates were observed under visible light, short wave UV light and long wave UV light. After development of plates, they were air-dried and number of spots, colour and Rf values were recorded.

$$Rf = \frac{\text{Spot distance from origin}}{\text{Solvent front distance from origin}}$$

RESULTS AND DISCUSSIONS

During the TLC different components were observed. Before reaching to most optimum solvent system a number of systems were employed.Chloroform crude extract was studied for thin layer chromatography (TLC) through different solvent systems. During the study TLC with solvent system Toluene: ethyl acetate: acetic acid (36:12:5) was most informative and showed five spots with R*f* values of values 0.4, 0.45, 0.46, 0.55, 0.82.

In the present study a number of developing solvent systems were tried during the study. The most informative and satisfactory resolution was taken as final solvent system. Plate was sprayed with detecting agent's i.e, anisaldehyde sulphuric acid. Solvent system Toluene: ethyl acetate: acetic acid (36:12:5) was found to be most appropriate and spotted TLC plate (Fig.) (silica gel G 60 F254 Merck, KGa A, 642721, Darmstadt, Germany) was sprayed with anisaldehyde sulphuric acid, heated at 110^0C for 5 minutes and observed under UV light. After development plates were dried and number of spots and R*f* values were recorded (Table).

$$Rf = \frac{\text{spot distance from origin}}{\text{Solvent front distance from the origen}}$$

Selection of mobile phase for TLC chloroform crude extract of *Solanum nigrum* L.

Extracts were analysed using thin layer chromatography to reveal most suitable solvent system suitable for separation of most of components present in extract. In randomly selected solvent system, Toluene: Ethyl Acetate: Formic acid (36:12:05), n-hexane: ethyl acetate: acetic acid (31:14:5) and n-hexane: ethyl acetate: formic acid (31:14:5) were found to be most effective in separation of components (Table). In which in first two solvent system provided maximum spots (12). From previous publications it was observed that these solvent system were used by in separation of components and they got good results using these solvent system.

Table: Showing TLC of different solvent systems:

Solvent system	Ratio	Total spots observed	R.f value
Ethyl acetate:Acetic acid:water	5:1:1	No spots	Nagative
Chloroform:Methnol:Water	7:4:1	2	0.88,0.91
n-hexane:ethyl acetate: Formic acid	31:14:5	5	0.64,0.67,0.80,0.85,0.92
n-hexane:ethyl acetate:Acetic acid	31:14:5	7	0.53,0.55,0.58,0.78,0.81,0.83,0.9
Chloroform:Methnol:Glacial acetic acid	50:40:0.5	Taling	Negative
Toluene:Ethyl acetate:Acetic acid	36:12:5	12	0.65,0.66,0.72,0.76,0.82,0.83,0.84,0.87,0.89,0.91,0.97,1

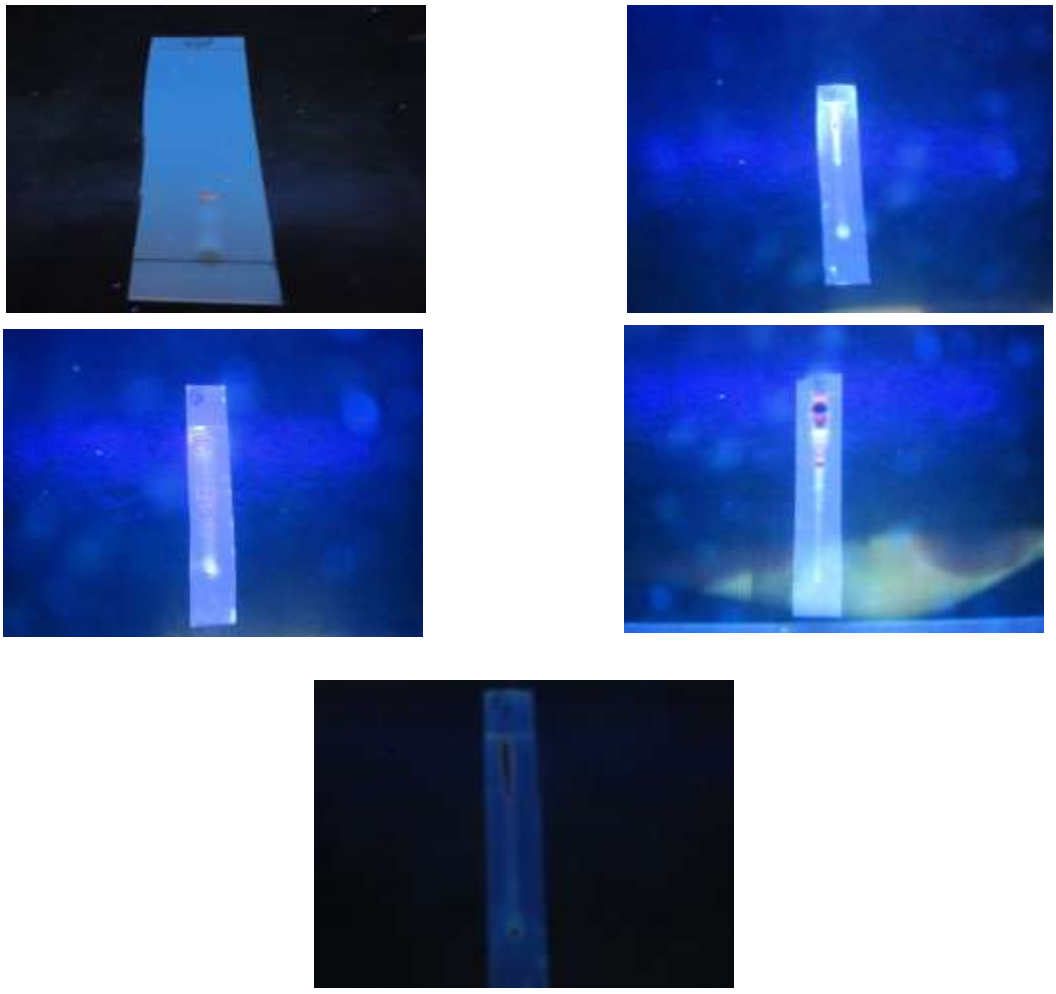

Fig: Thin Layer Chromatography (TLC) of Chloroform extract of *Solanum nigrum* L.

CONCLUSION

Thin layer chromatography is simple, cost-effective and easy to operate technique in phytochemistry and biochemistry with numerous applications which use in the development of new drugs and various types of formulations from medicinal plants. Further needed detailed documentation for the sustainable development in education and research.

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