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QUALITATIVE DETERMINATION OF BIOLOGICALLY ACTIVE CONSTITUENTS IN MEDICINAL PLANT CRUDE EXTRACTS BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT:
TLC is an analytical technique used for the qualitative and quantitative evaluation of Plant extracts. Qualitative determination by thin-layer chromatography of the biologically active and/or characteristic constituents of dried hydroethanolic or ethanolic extracts of the following three medicinal plants is reported: *Emblica officinalis Gaertn.*, *Withania somnifera L., Ocimum sanctum L.* The chromatographic separation was carried out on silica gel 60 F_{254} TLC plates. Toluene : Ethyl acetate : Acetic acid : Formic acid (20:45:20:5, v/v/v/v), Hexane : Isopropanol (8:2, v/v), Toluene : Ethyl acetate (9.9:0.1 v/v) as the mobile phase. Detection was carried out with keeping in Iodine chamber, under UV light or Dragendorff's reagent and Vanillin Sulphuric acid. The method is rapid, simple, and suitable for routine quality-control analysis of plant extracts.

Keywords: Plant extracts, *Emblica officinalis, Withania somnifera, Ocimum sanctum*, TLC.

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INTRODUCTION

Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products[1]. TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate)[2, 3]. Chromatography works on the principle that different compounds will have different solubility and adsorption to the two phases between which they are to be partitioned[4,5]. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent the stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed onto the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate[6,7]. The different compounds in the sample mixture move through the stationary phase at different rates, due to the different attractions for the mobile an stationary phases. Thus, individual compounds in the mixture separate as they move through the stationary phase. The separate compounds can be collected or detected, depending on the particular chromatographic technique involved[8, 9]. Amla pericarp, Withania root and Tulsi aerial parts were used for extract preparation[10].

MATERIALS AND METHOD

Procurement of plant materials

Dried fruits of Amla, dried roots of Withania and dried aerial part of Tulsi were procured from yucca enterprises Mumbai.

Preparation of extracts[11]

Procured dried plant materials (Amla pericarp, Withania root and Tulsi aerial part) were washed twice with tap water and one time with distilled water finally allowed to dry under shade, and then coarsely powdered in a blender. The coarse powder (500 gm each) was subjected to maceration for 72 hours, followed by exhaustive maceration for 48 hours by using various solvents like 60% ethanol for Amla pericarp, 50% ethanol for Withania root and 99.9% ethanol for Tulsi aerial part. The solvents were decanted and filtered with filter paper and recovered by distillation with help of rotary vacuum evaporator at 70^0C to 80^0C. The extracts were dried under desiccators.

Development of the optimum mobile phase[12]

The TLC procedure was optimized with a view to develop an assay method. The test solutions were spotted on TLC plates and different individual solvents as well as combination of solvents were tried to get a good separation. The extracts of these three plants were spotted on the TLC plates and run in different solvent systems. The above mentioned ratio gave good resolution Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

TLC procedure[13]

TLC was performed on a pre-coated TLC plates silica gel 60 F_{254} (20 cm × 5 cm) plate of 0.20 mm layer thickness. Chromatography was carried in twin through chamber which was pre-saturated with 10 ml mobile phase Toluene: Ethyl acetate: Acetic acid: Formic acid (20:45:20:5)v/v ratio for Amla ext., Hexane : Isopropanol (8:2) for Withania ext. and Toluene : Ethyl acetate (9.9:0.1) for Tulsi ext. separately for 30 min at room temperature (25 ± 2°C).

The samples were applied on the plates 10 mm from the bottom and chromatogram developed up to $3/4^{th}$ portion of TLC plates. Then plates were removed from TLC chamber and dried at room temperature. Amla spots were visualized by keeping in Iodine chamber, Withania spots were visualized by spraying with Dragendorff Reagent and Tulsi spot were visualized by spraying with vanillin Sulphuric acid reagent. Finally, the retention factor, or R_f, is calculated as the distance traveled by the compound divided by the distance traveled by the solvent[12].

RESULTS AND DISCUSSION

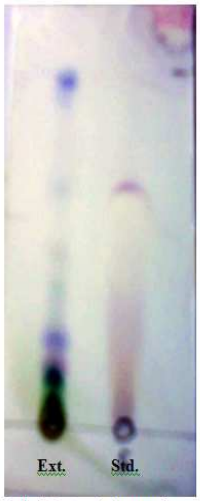
The presence of gallic acid in Amla and eugenol in Tulsi are conformed by TLC profile with help of standard drugs. (Fig.1, 2 & 3). Rf value of crude extract of amla showed presence of ascorbic acids and Tanins. Due to the presence of alkaloids, crude extract of withania showed by Rf value. The presence eugenol, ß-caryophylline showed by Rf value in Table 1.

CONCLUSION

In conclusion, the TLC method was found to be specific and accurate and can be used for qualitative estimation of crude extracts. TLC profiling of plant extracts in different solvent system confirms the presence of diverse group of phytochemicals.

Table: The Rf value of various extracts

S. No	Crude Extract	Solvent system	Detection	Rf
1.	Amla	Toluene:Ethylacetate: Aceticacid:Formicacid 20 : 45 : 20 : 5	Iodine chamber	0.25 0.69
2.	Withania	Hexane : Isopropanol (8:2)	U.v lamp or Dragendorff Reagent	0.54 0.88
3.	Tulsi	Toluene : Ethylacetate (9.9:1)	Vanillin sulphuric acid	0.38 0.97 0.32 0.26



Tulsi Ext. Eugenol

Fig. 1: Tulsi Ext.

Withania Ext.

Fig. 2: Withania Ext.

Amla Ext. Gallic acid

Fig. 3: Amla Ext.

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