### ABSTRACT

**Background:** Phenolic compounds especially the flavonoids and tannins comprise one of the most significant classes of plant secondary metabolites. **Aim:** HPTLC is widely accepted by WHO and pharmacopoeia across the globe as an effective analytical method for the investigation of phenolics in herbal analyses. **Materials and Methods:** The methanolic extracts of *Abrus precatorius* L., *Sapindus trifoliatus* L. and *Embelia ribes* Burm. F. were developed on the HPTLC system to study the diversity of phenolic compounds like flavonoids and tannins under different development conditions. Flavonoids were separated using the combination of solvent system comprising of mid-polar to polar solvents, hence Ethyl acetate: formic acid: glacial acetic acid: water (10:0.5:0.5:1 v/v/v/v) solvent mixture and 10% methanolic Sulphuric Acid Reagent was used as spray reagent for separation of flavanoids. Tannins were separated on chromatogram by using the solvent system comprising of mid-polar to non-polar solvents, hence Toluene: Ethyl Acetate: Formic Acid (6:4:0.3 v/v/v) as solvent system and alcoholic FeCl₃ was used for derivatization. **Results:** Distinct fingerprint of flavanoids and tannins was obtained. A profile of 11 to 12 polyvalent phytoconstituents were separated during the HPTLC analysis of flavonoids and tannins respectively. **Conclusion:** The HPTLC analysis successfully demonstrated that phenolic secondary metabolites can be effectively separated using the same extract under different development conditions. The detection and profiling of such metabolites provides the justification for therapeutic activities in medicinal plants. **Key words:** HPTLC, Flavonoids, Tannins, *Abrus precatorius* L., *Sapindus trifoliatus* L., *Embelia ribes* Burm. F.

### INTRODUCTION

Phenolic compounds encompassing a wide range of phytochemicals consisting of an aromatic ring with one or more hydroxyl groups. These compounds are mainly synthesized via the shikimate pathway but the polyketide pathway is also responsible for the biosynthesis of some phenolics.¹ Flavanoids forms the largest group among the phenolics and are characterized by the presence of two benzene rings which are joined by a propane unit. Tannins are polymers of polyphenols that are classified into two types, i.e., hydrolyzable and condensed tannins. HPTLC is widely used in the evaluation of diversity of various class of secondary metabolites from the same plant extract as well as for the development of characteristic chemical fingerprint of the entire plant extract.² ³ Literature review suggests that it is possible to achieve the separation of desired class of secondary metabolites from the same extract by choosing appropriate solvent system during the HPTLC analysis.⁴ ⁶ The present investigation were carried out by selecting appropriate solvent system for screening of flavanoids and tannins from medicinal plants *Abrus precatorius* L., *Sapindus trifoliatus* L. and *Embelia ribes* Burm. F. These plants are reported to exhibit antifertility activity and are quite common in Western Ghats region of Maharashtra.⁷
MATERIALS AND METHODS

Preparation of extracts

1% methanolic extract of air dried pericarp of *Sapindus trifoliatus* L., berries of *Embelia ribs* Burm. F. and seeds of *Abras precatorius* L. was Sonicated for 15 min in a bath sonicator without interval.

HPTLC fingerprinting

The HPTLC analyses was performed on aluminium plates pre-coated with silica gel 60 F$_{254}$, Merk, Germany. 1µL, 2µL and 5µL of extract was applied on the plate of 20 X 10 cm as bands of 8 mm width with the help of CAMAG Linomat V sample applicator. The plates were developed in a CAMAG Automatic Development Chamber 2 (ADC2) which was previously equilibrated with a mobile phase for 20 min. Flavonoids were separated using the solvent system of ethyl acetate: formic acid: galcial acetic acid: water (10:0.5:0.5:1 v/v/v/v). 10% methanolic Sulphuric Acid Reagent was used as spray reagent. Tannins were separated on chromatogram by using Toluene: Ethyl Acetate: Formic Acid (6:4:0.3 v/v/v) as solvent system and alcoholic FeCl$_3$ was used for derivatization (Wagner, Eike Reich). The plate was developed up to 8 cm, air dried, viewed and scanned at wavelength of 254 and 366 nm using CAMAG TLC Scanner 4 and CAMAG Visualizer 2. The plate was then derivatized in Automated CAMAG Derivatizer at level 3. The plate was heated at 105°C on CAMAG Plate heater till the development of colour. Derivatized chromatogram was again scanned at 254, 366 and 540 nm using CAMAG vision CATS software.

RESULT AND DISCUSSION

The extracts of *Abras precatorius* L., *Sapindus trifoliatus* L. and *Embelia ribs* Burm. F. Were subjected to generate HPTLC finger printing profile represented as chromatogram. The solvent system used in the investigation was found to give compact spots for extracts at different R$_f$ values and there was no overlap with any other component in the analysed sample. The spots were best observed at 366 nm after derivatization (Figure 1 and 2: HPTLC profile of Flavonoids in 1µL, 2µL and 5µL extracts of *A. precatorius, S. trifoliatus* and *E. ribs* at 366 nm after derivatization; Figure 3 and 4: HPTLC profile of Tannins in 1µL, 2µL and 5µL extracts of *A. precatorius, S. trifoliatus* and *E. ribs* at 366 nm and 540 nm after derivatization).

The results from HPTLC finger print scanned at wavelength 366 nm 2µL extract of *A. precatorius* for flavonoids (Figure 5 Densitogram and corresponding R$_f$ values of 2µL extract of *A. precatorius* for
flavonoids) reveal the occurrence of 11 polyvalent phytoconstituents with corresponding \( R_f \) values of 0.04, 0.12, 0.15, 0.26, 0.38, 0.53, 0.57, 0.74, 0.80, 0.89 and 1.00. As shown in Figure 1 out of 11 components, the component with \( R_f \) values 0.26, 0.38, 0.89 and 1.00 were found to be more predominant as the percentage area was more with 10.23%, 5.47%, 14.43% and 54.61% respectively. Among these the highest concentration of the phytoconstituents was found to be 54.61% and its corresponding \( R_f \) value was found to be 1.00. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 5.0%.

The results from HPTLC finger print scanned at wavelength 366 nm for 2µL \( E. \) ribes (Figure 6). Densitogram and corresponding \( R_f \) values of 2µL extract of \( E. \) ribes for flavanoids) reveal the occurrence of 8 polyvalent phytoconstituents and corresponding ascending order of \( R_f \) values is 0.02, 0.16, 0.39, 0.50, 0.77, 0.87, 0.91 and 0.97. As shown in Figure 6, 6 out of 8 components, the component with \( R_f \) values 0.02, 0.16, 0.39, 0.50, 0.87, 0.91 and 0.97 were found to be more predominant as the percentage area was more with 10.41%, 19.21%, 6.34%, 8.33%, 11.62%, 9.59% and 33.72% respectively. Among these the highest concentration of the phytoconstituents was found to be 33.72% and its corresponding \( R_f \) value was found to be 0.97. The remaining components was found to be very less in quantity as the percent area for the spot was less than 5.0%.

The results from HPTLC finger print scanned at wavelength 366 nm for 5µL \( S. \) trifoliatus (Figure 7 Denstogram and corresponding \( R_f \) values of 5 µL extract of \( S. \) trifoliatus for flavanoids) reveal the occurrence of 11 polyvalent phytoconstituents and corresponding ascending order of \( R_f \) values is 0.01, 0.10, 0.18, 0.22, 0.30, 0.47, 0.53, 0.64, 0.80, 0.85 and 0.96. As shown in Figure 7, 3 out of 11 components, the component with \( R_f \) values 0.10, 0.18, 0.22, 0.80, 0.85 and 0.96 were found to be more predominant as the percentage area was more with 9.83%, 12.12%, 14.37%, 5.4%, 8.15%, 8.27% and 28.40% respectively. Between these the highest concentration of the phytoconstituents was found to be 22.75% and its corresponding \( R_f \) value was found to be 0.96. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 5.0%.

Flavonoids comprise a large group of plant secondary metabolites characterized by a Diphenyl propane structure (C6-C3-C6). They are widely distributed throughout the plant kingdom and are commonly found in fruits, vegetables and certain beverages. Numerous preclinical and some clinical studies suggest that flavonoids have potential for the prevention and treatment of several diseases like free radical scavenging and enzyme inhibitory activities, antioxidant effects, antibacterial effect, anti-cancer effect, cardio protective effects, immune system promoting and anti-inflammatory effects, skin protective effect from UV radiation, Alzheimer's and Parkinson's diseases\(^8\)-\(^11\)
print for Tannins scanned at 540 nm wavelength for 5µL. *E. ribes* (Figure 9. Densitogram and corresponding *R* values of 5µL extract of *E. ribes* for Tannins) reveal the occurrence of 9 polyvalent phytoconstituents and corresponding ascending order of *R* values is 0.06, 0.10, 0.22, 0.31, 0.49, 0.60, 0.70, 0.81 and 0.98. As shown in Figure 9.5 out of 9 components, the component with *R* values 0.22, 0.60, 0.70, 0.81 and 0.98 were found to be more predominant as the percentage area was more with 10.41%, 19.21%, 6.34%, 8.33%, 11.62%, 9.59% and 33.72% respectively. Among these the highest concentration of the phytoconstituents was found to be 0.57% and its corresponding *R* value was found to be 0.98. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 5.0%.

Tannins belong to polyphenolic compounds and available in various plant parts like leaves, roots, stem bark, flower and fruits and largely distributed in various species of higher plants. Tannins play various roles in higher plants such as provide protection against predation, act as pesticides, antioxidant, free radical scavenging activity, anti-microbial, anti-cancer, anti-nutritional anti-bacterial and anti-fungal activity. It also shows cardio-protective, hepatoprotective and hypoglycemic properties. 12-14

CONCLUSION

Herbal drugs are a complex mixture of active metabolites. Bioactivity of herals is not entirely due to the presence of any single active principle but is dedicated to the presence of a wide array of complex metabolites often working synergistically. HPTLC allows the profiling of various classes of secondary metabolites from the same or different plants by choosing the solvent system of varying polarity. Multiple fingerprints from different plant sources can be simultaneously developed, each representing the profile of the same class of compound. The present study demonstrates the utility of HPTLC for studying each class of phenolic compounds from three different sources on the same plate. Diversity in flavonoids and tannins can be further worked upon to evaluate the bioactivity of individual phenolic compounds for their pharmaceutical application.

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CONFLICT OF INTEREST

Authors hereby declare that there is no conflict of interest

ABBREVIATIONS

HPTLC: High Performance Thin Layer Chromatography; 
µL: Micro litre; nm: Nano meter; *R* : Retardation factor; 
v/v: Volume by volume.
REFERENCES


PICTORIAL ABSTRACT

SUMMARY

The present study led to the development of fingerprint profile of phenolics viz. flavonoids and tannins by HPTLC. The medicinal plant material were sonicated and subjected to the HPTLC studies according to the ICH guidelines. The solvent system designed selectively separates flavonoids and tannins fraction from the analyte on the basis of their selective differential coefficient. The solvent system of Ethyl acetate: formic acid: galcial glacial acetic acid: water (10:0.5:0.5:1 v/v/v/v) was found effective for the separation of flavonoids. The fingerprint was best viewed at 366 nm after the derivatization with 10% methanolic sulphuric acid. Tannins were separated using the solvent system of Toluene: Ethyl Acetate: Formic Acid (6:4:0.3 v/v/v). The fingerprint was best viewed at 540 nm after the derivatization with alcoholic ferric chloride reagent as derivatizing agent. The present study could be used as a tool for rapid identification and separation of flavonoids and tannins from any medicinal plant using HPTLC.
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