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HPTLC Profile Of Methanolic Extract Of Achyranthus Aspera Stem And Leaves

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The present study aimed to establish the High-Performance Thin-Layer Chromatography (HPTLC) profile of the methanolic extract of Achyranthes aspera L. stem and leaves to aid in the standardization and quality evaluation of the plant material. Freshly collected stem and leaves were shade-dried, powdered, and extracted with methanol using a Soxhlet apparatus. The resulting extracts were subjected to HPTLC analysis using silica gel 60 F_{254} plates as the stationary phase and a suitable mobile phase system optimized for maximum resolution of phytoconstituents. The developed chromatograms were visualized under UV light at 254 nm and 366 nm, as well as after derivatization with anisaldehyde-sulfuric acid reagent, to detect and document phytochemical fingerprints. The comparative chromatographic profile highlighted both shared and unique phytochemical markers in the two plant parts. The HPTLC fingerprint generated in this study can serve as a rapid, reliable, and reproducible tool for the authentication, phytochemical evaluation, and quality control of Achyranthes aspera in herbal formulations.

Key-words: Achyranthes aspera, Extract, HPTLC

Introduction

Achyranthes aspera L., commonly known as Apamarga or prickly chaff flower, is an important medicinal plant belonging to the family Amaranthaceae and is widely distributed in tropical and subtropical regions of Asia, Africa, and Australia. Traditionally, it has been used in Ayurveda, Unani, and folk medicine for its diverse therapeutic properties. The plant is rich in phytochemicals such as alkaloids (achyranthine), saponins, flavonoids, tannins, glycosides, steroids, and triterpenoids, which contribute to its wide range of biological activities. The seeds contain ecdysterone, while the roots are known to possess alkaloids and saponins with notable medicinal value. Pharmacologically, Achyranthes aspera exhibits anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, anti-fertility, analgesic, diuretic, and immunomodulatory properties, along with activity against cardiovascular and metabolic disorders. It has also been reported to aid in wound healing, asthma, hypertension, diabetes, and digestive ailments. Due to this rich phytochemistry and broad pharmacological profile, Achyranthes aspera continues to be a plant of great interest in ethnomedicine and modern drug discovery research. [1-2]

High-Performance Thin Layer Chromatography (HPTLC) analysis plays a crucial role in the standardization, quality control, and authentication of medicinal plants such as Achyranthes aspera. Being a simple, reliable, and cost-effective analytical technique, HPTLC allows for the separation, identification, and quantification of multiple phytoconstituents in a single run, thus providing a comprehensive chemical fingerprint of plant extracts. For Achyranthes aspera, which is known to contain diverse bioactive compounds like alkaloids, saponins, flavonoids, and glycosides, HPTLC is particularly important in detecting and differentiating these constituents to ensure consistency in herbal formulations. It helps in identifying marker compounds, monitoring batch-to-batch variations, and detecting adulteration or substitution with other plant materials. Moreover, HPTLC offers advantages such as high sample throughput, minimal sample preparation, and suitability for both qualitative and quantitative analysis, making it highly valuable for pharmacognostic and phytochemical studies. The importance of HPTLC analysis of Achyranthes aspera therefore lies in its ability to establish reliable standards, ensure therapeutic efficacy, and support regulatory acceptance of herbal drugs derived from this plant. [3-5]

Material and Methods

Chemicals and reagents

All chemicals and reagents used in the study were of analytical grade and were purchased from reliable firms and institutes (CDH, MERCK, and SUYOG). Silica gel 60 F254, HPTLC aluminium sheets 10 x 10 cm, Merck KGaA, Germany.

Instrumentation

A CAMAG Linomat 5 executed by anchrom HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4, twin trough plate development chamber, TLC Scanner 3, win CATS software version 1.4.4. and Hamilton (Reno, Nevada, USA).

Development of HPTLC finger print profile of Achyranthus aspera

High-performance thin layer chromatography (HPTLC) analysis was performed for four studies samples with standard methods applied. [6-7]

HPTLC densitometric estimation of (Ferulicacid, Quercetin and Stearicacid)

Preparation of methanol extract of plant sample solution

The methanol extract of the plant was used for HPTLC analysis. High-performance thin layer chromatography (HPTLC) studies were performed as per the method opted by Rajput et.al.2012, and Tripathiet.al.2014.Take 50 mg powder of leaves and stem then, extracted with 5ml of methanol solution overnight and filter, after the filtration concentra ted plant sample was applied on precoated silica gel aluminum plate F-254 (0.2mmthickness) Merck, Mumbai, using Camag lino mat-5 sample applicator Camag syringe. For High-performance thin layer chromatography about 5 gm accurately weighed Achyranthus aspera samples powde r(leaves and stem) with 100 ml of methanol (3X100) in a Soxhlet apparatus for 6 hours separately. Filtered and concentrated the extracts under a vacuum to get the residue. In a volumetric flask dissolve 100 mg of Achyranthus aspera extracts in 10ml (10mg/ml) and make up the volume with methanol to get the working test solution separately.

Preparation of Standard marker Solution-(Ferulicacid, Quercetin and Stearicacid)
In a 10 ml volumetric flask, 10 mg of ferulic acid, quercetin, and stearic acid were dissolved to create standard marker working solutions. The remaining volume was filled with methanol on its own. Next, add 0.1 mg/ml of methanol to a 10 ml volumetric flask to make up the volume after transferring 1 ml of the stock solution there. Standard solutions were generated from the solution by adding methanol solution to 10 ml volumetric flasks in increments of 0.1, 0.2, 0.3, and 0.4ml, which corresponded to 1, 2, 3, and 4 ug/ml of the stock solution.

Solvent system- Toluene:Ethylacetate (7:3v/v)

Results and Discussion
HPTLC profile of methanol extract of Achyranthus aspera Stem and Leaves along with Toluene: ethyl acetate (7:5v/v) as the mobile phase determined major spots at different Rf before derivatization and after derivatization at 254nm, and 366nm respectively. Standard biomarkers Ferulic acid, Quercetin, and Stearic acid were employed for the determination of secondary phytoconstituents.
From Table No 1 the densitometric analysis of Achyranthus aspera was performed at 254 nm in reflectance mode. The Rf values of the ferulic acid marker compounds were in the range of 0.28 to 0.77. The Rf values of the Quercetin marker compounds were in the range of 0.77 to 0.8 while the Rf values of the stearic acid marker compounds were not detected. The stem extract of Achyranthus aspera at 254 nm shows the existence of Quercetin phenolic compound with one spot with Rf values 0.8 (light black) similar to standard Rf values. Whereas leaf extract of Achyranthus aspera at 254 nm shows the presence of stearic acid with two spots with Rf values 0.8 (light black), and 0.28(light black) similar to standard Rf values. From Table No. 2 the densitometric analysis of Achyranthus aspera was performed at 366 nm in reflectance mode. The Rf values of the ferulic acid marker compounds were in the range of 0.3-0.6. The Rf values of the Quercetin marker compounds were in the range of 0.1 to 0.9 while the Rf values of the stearic acid marker compounds were not detected.
When the plates were viewed at 366nm after derivatization shows the presence of a ferulic acid phenolic compound with two spots with Rf values 0.6 (reddish brown) and 0.9 (blue) similar to standard Rf values. Stem extract of Achyranthus aspera at 366 nm shows the presence of Quercetin phenolic compound with two spots with Rf values 0.7 (blue black) and 0.9 (sky blue) similar to standard Rf values. Whereas leaf extract of Achyranthus aspera at 366 nm shows the presence of stearic acid with one spot with Rf values 0.9 (reddish) similar to standard Rf values.

Table 1: Rf values of HPTLC fingerprints profile of Achyranthus aspera at 254nm

Rf Value	Ferulic acid	Quercetin	Stearicacid	Stem	Leaf
Rf1	0.28	0.77	ND	0.8	0.8
Rf2	0.77	0.8	ND	-	0.28

Table 2: Rf values of HPTLC finger prints profile of Achyranthus aspera at 366nm

Rf Value	Ferulic acid	Quercetin	Stearic acid	Stem	Leaf
Rf1	0.3	0.9	ND	0.7	0.9
Rf2	0.6	0.1	ND	0.9	ND

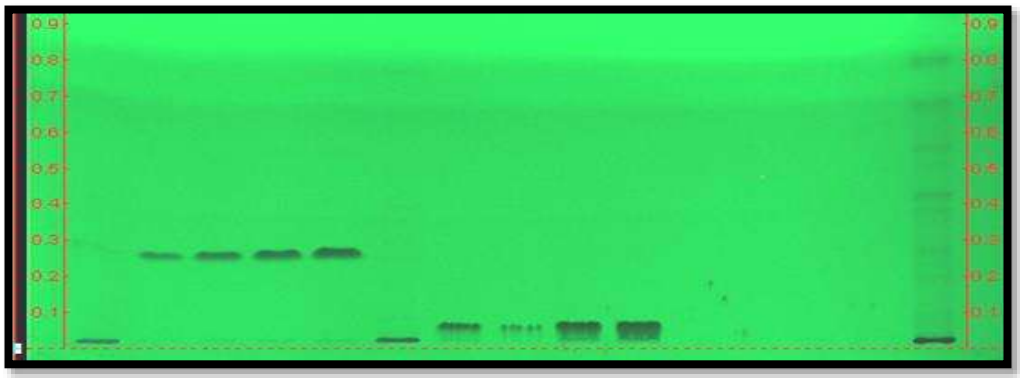
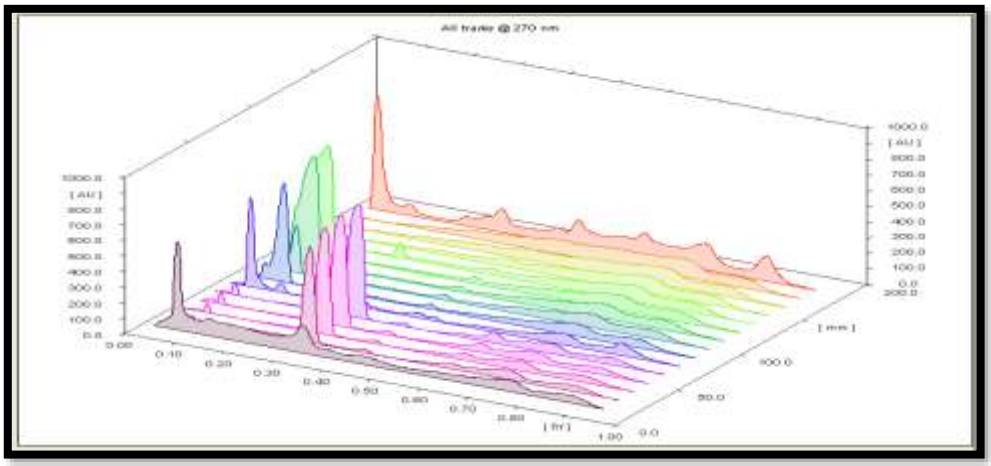


Fig 1: HPTLC of Achyranthus aspera

Where**, Track 1-5:** Ferullic acid standard; **Track 6:**Test solution of **Achyranthus aspera stem; Track 7-10:** Stearic acid; **Track 11-14:** Stearic acid standard; **Track 15:** Test solution of **Achyranthus aspera leaf.**

Fig. 2: 3D Graph Densitometric HPTLC chromatogram of standards Quercetin,



Ferulicacid & Stearic acid of Achyranthus aspera stem and leaf aqueous extracts

Conclusion

The present study on the HPTLC profile of the methanolic extract of Achyranthes aspera stem and leaves concludes that the plant contains a diverse range of phytoconstituents which can be effectively separated and identified through this technique. The developed HPTLC fingerprint serves as a reliable tool for the qualitative and quantitative evaluation of bioactive compounds, thereby ensuring the authenticity, purity, and standardization of the plant material. The observed chromatographic bands highlight the presence of multiple phytochemicals such as flavonoids, alkaloids, and saponins, which correlate with the known pharmacological properties of Achyranthes aspera. This study not only validates the traditional use of the plant in herbal medicine but also provides a scientific basis for its inclusion in modern formulations. Overall, the HPTLC fingerprint generated in this research can be used as a reference standard for quality control and future phytochemical or pharmacological investigations of Achyranthes aspera.

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