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HPTLC FINGERPRINTING FOR SIMULTANEOUS QUANTIFICATION OF GALLIC ACID, QUERCETIN AND GLYCYRRHIZIN IN THE METHANOLIC EXTRACTS OF *ABRUS PRECATORIUS* AND *CORDIA WALLICHI* LEAVES AND ITS FORMULATION

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ABSTRACT

A simple, accurate and reproducible HPTLC method has been developed for the simultaneous quantification of Gallic acid, Quercetin and Glycyrrhizin in the methanolic extracts of *Abrus precatorius* (MEAP) and *Cordia wallichi* leaves (MECW). The method was successfully applied for quantification of three biomarkers in the formulations prepared from methanolic extracts of *Abrus precatorius* and *Cordia wallichi* leaves. The CAMAG HPTLC systems with Win CATS;Version 1.4.9 software used for the analysis. Double development with mobile phase toluene: ethyl acetate:formic acid:methanol (4.5: 3: 1: 1.5 v/v/v/v) on TLC plate silica gel 60 F_{254}. Detection and quantification of Gallic acid (GA), Quercetin (QUE) and Glycyrrhizin (GLY) were carried out before derivatization with anisaldehyde sulphuric acid reagent. The quantity of Gallic acid, Quercetin and Glycyrrhizin in MEAP was found to be 0.59%, 0.15%, 0.11% and the in MECW the content of gallic acid and quercetin was found 0.35% and 0.23% respectively. In the Polyherbal floating tablet, the quantity of Gallic acid, Quercetin and Glycyrrhizin equivalents (GAE, QE and GE) were found to be 0.46%, 0.19%, 0.05% respectively. The developed method can be successfully applied for

quantification of these three components in any formulation containing *Abrus precatorius* and *Cordia wallichi* leaf extract.

Keywords: Simultaneous quantification, HPTLC, Gallic acid, Quercetin, Glycyrrhizin, *Abrus precatorius*, *Cordia wallichi*

1. INTRODUCTION

Many Developed countries also suffer a big problem in standardizing and quality control of the herbal plants. This is due to lot many factors among which are the complex form of the plant constituents and the inability of the traditional methods to precisely estimate the quality of the herbs **[1]**.

The quality of herbal medicines is evaluated on the content of it's bioactive compounds. Technological advancements which take place in the processes of isolation, purification and structural elucidation of natural compounds have made it probable to generate appropriate strategies for the analysis of quality and standardization of plant based medicines **[2]**.

Chromatographic fingerprint investigation has been demonstrated to be a realistic and practical method for quality assessment and species authentication of various traditional medicines. HPTLC chromatogram pattern comparison seems to be promising for fingerprinting the active compounds in plant extracts. TLC and HPTLC are methods commonly applied for the identification, the assay and the testing for purity, stability, dissolution or content uniformity of raw materials **[2]**.

Modern high-performance TLC (HPTLC) is an efficient instrumental analysis, and optimized quantitative HPTLC using a Densitometric evaluation can produce results analogous to those obtained with gas chromatography (GC) and high performance liquid chromatography (HPLC**) [2, 3]**.

Among the traditional system of medicine *Abrus precatorius* Linn. (Fabaceae) commonly known as Indian Liquorice, is a climbing shrub found in subtropical regions of India. It is a beautiful deciduous, climbing plant. Its seeds have remarkably uniform weight of 1/10th of a gram, Hence, were used by goldsmiths as standard weights for weighing gold and silver in previous time. The plant is used in some traditional medicine to treat scratches, sores and wounds caused by dogs, cats and mice, and are also used with other ingredients to treat leucoderma, tetanus and rabies. They are ground with lime and applied on acne sores, boils and abscesses. The seeds are considered abortifacient **[4, 5]**. Abrus precatorius is rich in various chemical constituents such as abrol, abrasine, precol

and precasine from the roots. Seeds are rich in several essential amino acids like serine, alanine, valine, choline and methyl ester **[6]**. Seeds are poisonous and contain principle compound Abrine, Abraline, Anthocyanins, Calcium, Campesterol, Choline, Cycloartenol, Delphinidin, Gallic-acid, Glycyrrhizin, Hypaphorine, N, N-dimethyl-tryptophan, N, N- dimethyl-tryptophan-metho-cation-methyl-ester.**[7]**

The leaves are used traditionally as nerve tonic, applied on cuts and swellings and mouth ulcer **[8]**. This plant is also reported as antidiabetic, antifertelity, antimalarial, antispasmodic, antibacterial and neuro-muscular blocking activity, Uterotonic activity **[9-14]**.

The identification and quantification of Phytoconstituents in various extracts, biological samples and formulations was addressed in some report **[15]**. The analytical methodology in these scientific reports involved the use of HPTLC-densitometric analysis **[16]** Reversed phase high performance liquid chromatography (RP-HPLC) **[17]**, free radical scavenging activity, **[18, 19]** on the leaves as well as seed of this plant.

About 300 species of genus *Cordia* have been identified worldwide. There are 13 species of this genus found in India. The plant parts like fruits, leaves, stem bark, seeds and roots of most species of plants of the genus *Cordia*, especially *Cordia dichotoma, C.myxa, C.oblique, C.verbenacea, C.martinicensis, C.salicifolia, C.spinescens, C.latifolia, C. ulmifolia*, among others, has long been used in traditional medicine . The leaf, stem and root powders with various extracts showed the presence of phenols, alkaloids, tannins and reducing sugar. Leaves used for ulcers and headaches. In India traditionally used for ulcerative colitis, ulcers, and colic pain **[20, 21]**.

Researchers reported pharmacognostical, physico-chemical, phytochemical, fluores-cence and HPTLC chromatographic profile of the leaves of *Cordia dichotoma* Linn. It revealed 3 peaks at UV 254 nm **[22-26]**. Although HPTLC fingerprints has been reported on the Chloroform extract of *Cordia wallichi* leaves [**27]** but no HPTLC fingerprints and estimation has been reported on Methanolic extract of *Cordia wallichi* leaves.

However, pertaining to our knowledge there is no any hyphenated HPTLC technique available anywhere else for simultaneous estimation of GA and QUE in methanolic extract of *Abrus precatorius and GA and QE in Cordia wallichi* leaves. So, the attempt has been made to accept this challenge towards development and validation of GA, QUE and GLY simultaneously by such a hyphenated technology like HPTLC for the betterment of herbal quality standards.

2. MATERIALS AND METHODS

2.1 Chemicals

Ethyl acetate, toluene, methanol and formic acid of analytical grade were purchased E.Merck, Mumbai, India. Anisaldehyde sulphuric acid reagent was prepared as per reported method. (Wagner). Reference standards of Gallic acid(GA), Quercetin(QUE) and Glycyrhizin(GLY) were purchased from Sigma-Aldrich GmbH, Germany. All other solvents and chemicals were of the highest analytical grade.

2.2 Apparatus

Linomat V Automatic Sample Spotter (CAMAG, Muttenz, Switzerland), 100 µL syringe (Hamilton, Bonaduz, Switzerland), glass twin trough chamber (20 cm × 10 cm × 4 cm) (CAMAG), TLC Scanner 3 linked to Win Cats software (CAMAG), 0.2 mm thickness pre-coated with silica gel 60 F254 (Merck) were used in this study. The experiment was carried out under the conditions with temperature of (25±2) °C and relative humidity of 40%.

2.3 Quantitative estimation of Gallic acid, Quercetin and Glycyrrhizin

2.3.1 Plant Material

The authentic plant materials *of Abrus precatorius* and *Cordia wallichi* leaves were collected from Kamrup district, Assam and identified and authenticated by Dr.T.G.Gohil, taxonomist and HOD of Botany, Botanist in B.K.M Science College, Valsad (Gujurat). Voucher specimen of the collected plants were prepared and maintained in the Botany department of BKM Science College for future reference. The plant were washed with water to remove any dust particles, dried in shade, powdered and then sieved through BSS mesh size 85 and stored at 25^{o}C in an airtight container.

2.3.2 Preparation of standard solutions of Gallic acid, Quercetin and Glycyrrhizin

1 mg/ml stock solutions of each three biomarkers were prepared in methanol. It was further diluted to 10µg/ml in methanol. Then each standard solution was mixed in equal proportion to make final working standard solution.

2.3.3 Preparation of sample solutions

A 20mg/10ml solution of the *Abrus* and *Cordia* leaves powder were dissolved in methanol. The methanol extract of the powder was prepared by sonication for 20 minutes. The extract was filtered through Whatman paper no.1.

2.3.4 Preparation of the formulation extract

Ten polyherbal tablets were powdered using mortar pestle.1gm of sample was weighed in a conical flask.85ml of methanol were added. Sonicated for 10 minutes followed by warming on water bath for 10 minutes. Supernatant was filtered through Whatman No. 1 filter paper

in a 100 ml volumetric flask. Volume was adjusted with methanol and 10 µl were injeced to HPTLC plate.

2.3.5 Procedure

Analysis was performed on 20 cm × 10 cm HPTLC silica gel G60 F_{254} plates with fluorescent indicator. Before starting the analysis, HPTLC plates were cleaned by predevelopment with methanol by ascending method. HPTLC plate was immersed in a CAMAG glass chamber (20 cm × 10 cm), containing 30 mL methanol (HPLC grade) as solvent system. The chamber was covered with glass lid and left till development of the plate to the top with methanol. After complete development, the plate was removed from TLC glass chamber and dried in an oven at 105 °C for 5 min). A constant application rate of 0.1µl/s was used and distance between two bands was 6 mm. Different volumes from 4-12µl of the solution were applied, which gave different concentration 400-1200ng per band respectively.

Standards bands of 10 µL were applied and analysed, the standard preparation along with the bands of sample preparation as the bands on the same plate by means of a CAMAG Linomat 5 (automated spray-on applicator equipped with a 100 µL syringe and operated with the settings band length 6 mm, distance between band 15 mm, distance from the plate side edge 15 mm, and distance from the bottom of the plate 15mm **[28-30]**.

2.3.6 Determination of analytical wavelength

Scanning was performed in the reflectance-absorption mode using a UV detector in the range of 200-400 nm. All the three markers showed reasonable good response at 254 nm. So they were detected at this analytical wavelength.

2.3.7 TLC development and scanning of Gallic acid, Quercetin and Glycyrrhizin

The plate was developed by immersing sample HPTLC plate in a CAMAG glass chamber (20 cm × 10 cm) containing Optimized solvent system, toluene: ethyl acetate:formic acid:methanol (4.5: 3: 1: 1.5 v/v/v/v). After complete development, the plate was allowed to dry by keeping in fume cupboard for 10 min and then kept in hot air oven for 5 min at 105 °C. The plate was scanned in the densitometer by linear scanning at 254 nm for GA, QUE and GLY by using a TLC Scanner V CAMAG with a D2 source, and integrated the area of the spots corresponding to Gallic acid, Quercetin and Glycyrrhizin. The R_f values of Gallic acid (0.66), Quercetin (0.79) and Glycyrrhizin (0.22) in both the samples and Reference Standard were found comparable under UV light at 254 nm. Peaks were symmetrical in nature and no tailing was observed when peaks were scanned at 254nm. The response of the sample

solution was measured at 254 nm for the quantification of GA, QUE and GLY by comparing average area of the sample and standard. The amounts of GA, QUE and GLY in both the plant extracts and it's formulation are calculated by the proposed method.

Amount of GA, QUE and GLY in both MEAP and MECW were calculated by following formula:

$$\frac{\text{ASMP}\times\text{WSTD}\times\text{Dilution of Smp}\times\text{Application vol of sample}\times\text{P}}{\text{ASTD}\times\text{Dilution of Std}\times W_{SMP}\times\text{Application of vol of standard}}$$

Where ASMP is average area of sample; ASTD is average area of standard; WSTD is weight of standard, mg; WSMP is weight of sample, g; Dilution of Smp is dilution of sample, mL; Dilution of Std is dilution of standard, mL; P is percent purity of standard (As per COA).

3. RESULTS AND DISCUSSION

In the current study quantitative estimation of specific biologically active Gallic acid, Quercetin and Glycyrrhizin components were conducted in the methanolic extracts of *Abrus precatorius* and *Cordia wallichi* leaves using HPTLC and it's formulation. For optimization of method, different mobile phase compositions were employed to achieve good separation. Among the various solvent systems tried the solvent system containing toluene: ethyl acetate:formic acid: methanol (4.5: 3: 1: 1.5 v/v/v/v) resulted in good separation of all the three markers. TLC plate was observed under UV light for the presence of GA, QUE and GLY which were detected by prominent dark brown spots. The R_f values of Gallic acid (**0.66**), Quercetin (**0.79**) and Glycyrrhizin (**0.22**) in both the samples, it's formulation and Reference Standard were found comparable under UV light at 254 nm. Peaks were symmetrical in nature and no tailing was observed when peaks were scanned at 254nm.

Different solvent systems were used for the detection of all the three markers simultaneously of which the solvent system containing toluene: ethyl acetate:formic acid: methanol (4.5: 3: 1: 1.5 v/v/v/v) resulted in good resolution of GA,QUE and GLY in the presence of other compounds in the plant extracts. **Figure 1** represents the Standard UV spectra overlay of the three biomarkers. **Figures 2 and 3** Photo Scan of TLC plate for the simultaneous estimation of GA, QUE and GLY at 254 nm and at 366 nm. **Figure 4** represented Photo Scan of TLC plate after derivatization with Anisaldehyde Sulphuric acid (ASA). **Figure 5** represents typical scan of TLC plate of Polyherbal formulation at 254 nm.

The separated band appeared for all the standards in the UV Visualizer which was scanned at 254nm with a CAMAG TLC Scanner with win CATS 5 software, using the Deuterium lamp.

An accurate, simple and specific HPTLC method for quantitative estimation of biomarkers present in the methanolic extracts of *Abrus precatorius* (MEAP) and *Cordia wallichi* leaves (MECW) has been developed. The method employed in current study resulted in good peak shape of standard Gallic acid, Quercetin and Glycyrrhizin establishing R_f values 0.66, 0.79 and 0.22 respectively represented at **Figure 6-9**, represents the Chromatograms of the Mixed standard biomarkers (1:1:1) in the optimized solvent system. **Figure 10, 11** representing the Chromatograms of biomarkers in the MEAP and MECW. **Figure 12** represents densitograms of Phytoconstituents in polyherbal tablet (Floating).

There was no interference from the other phytoconstituents present in both the extracts. Hence the quantity of Gallic acid, Quercetin and Glycyrrhizin in MEAP was found to be 0.59%, 0.15%, 0.11% and the in MECW the content of Gallic acid and Quercetin was found 0.35% and 0.23% respectively. In the polyherbal Floating tablet, the quantity of Gallic acid, Quercetin and Glycyrrhizin were found to be 0.18%, 0.07%, 0.02% respectively.

A normal phase high performance thin layer chromatographic (HPTLC) method for the simultaneous quantification of Gallic acid, Quercetin and Glycyrrhizin in the methanolic extracts of *Abrus precatorius* and *Cordia wallichi* leaves and it's formulation was developed in the present research work.

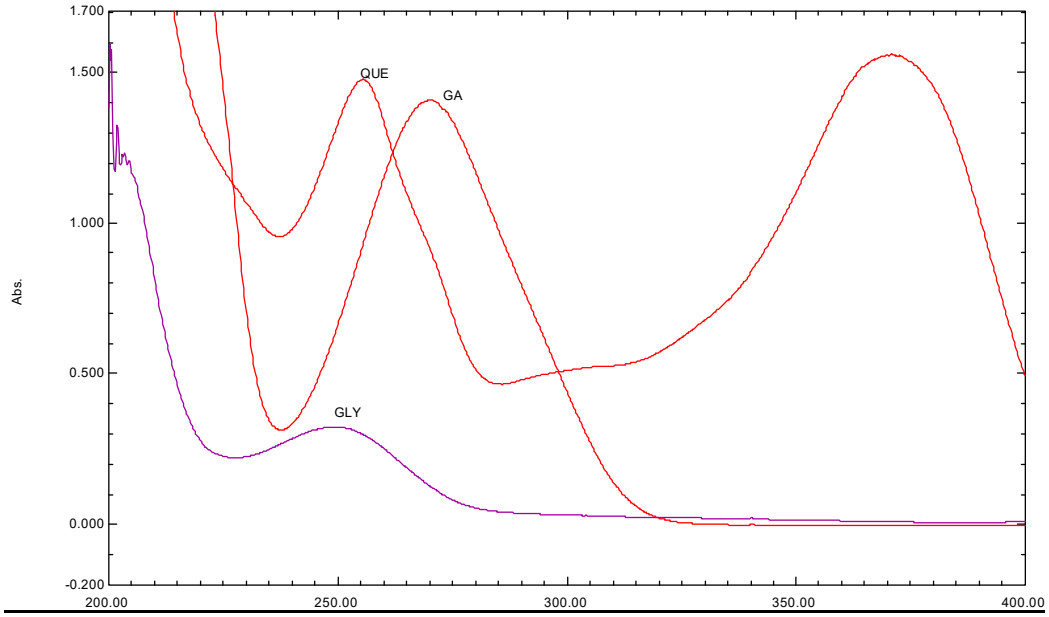

Figure 1: Standard spectra overlay of GA, QUE and GLY biomarkers at 254nm

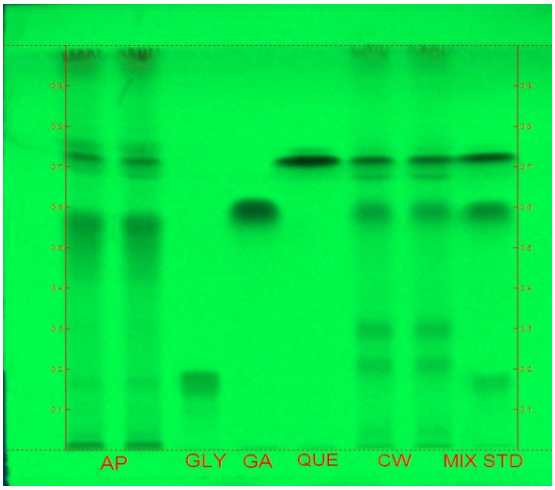

Figure 2: Typical scan of tlc plate at at 254nm

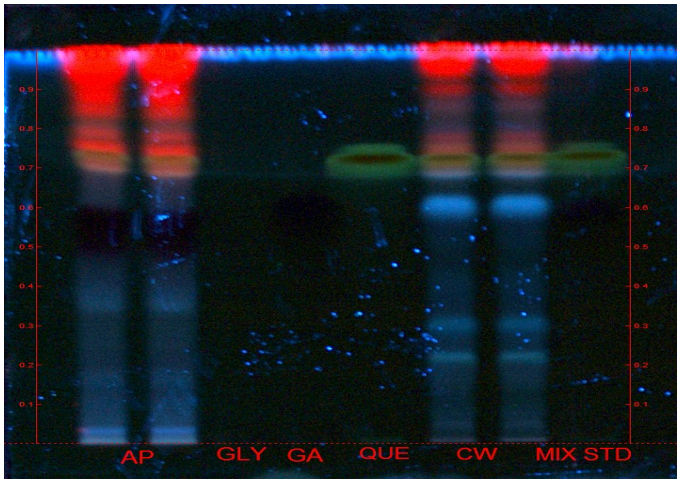

Figure 3: Typical scan of tlc plate at 366nm

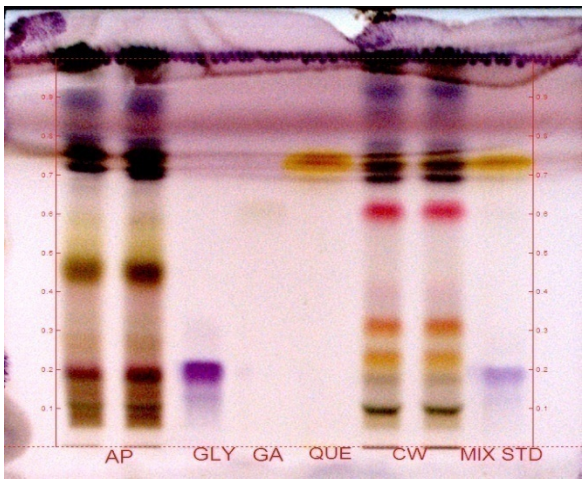

Figure 4: Typical scan of TLC Plates After Derivatization with ASA reagent

Figure 5: Typical Scan of TLC Plate of Polyherbal Formulation at 254 nm

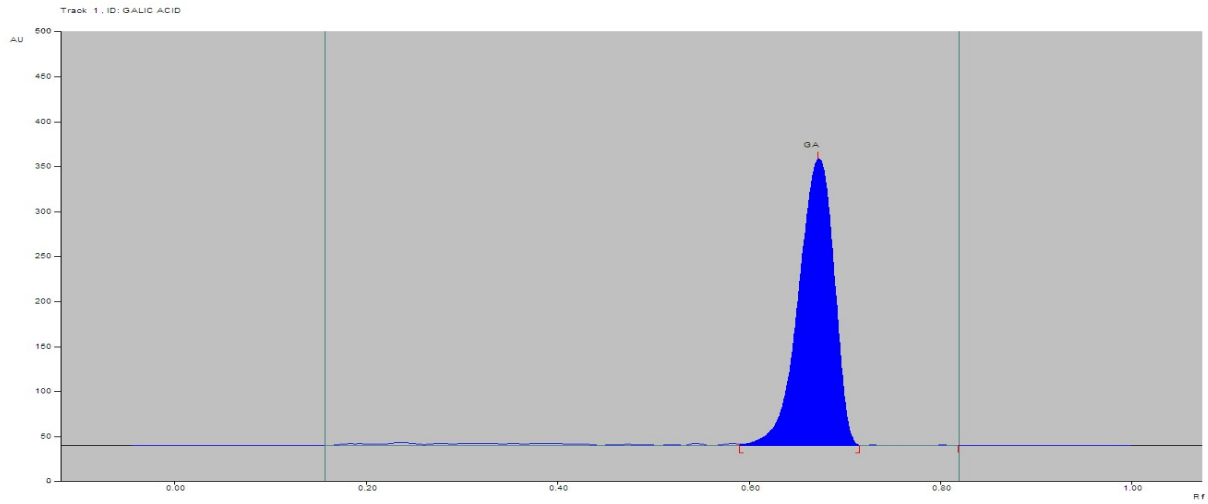

Figure 6: Chromatograms of Standard Galic Acid at Rf 6.6

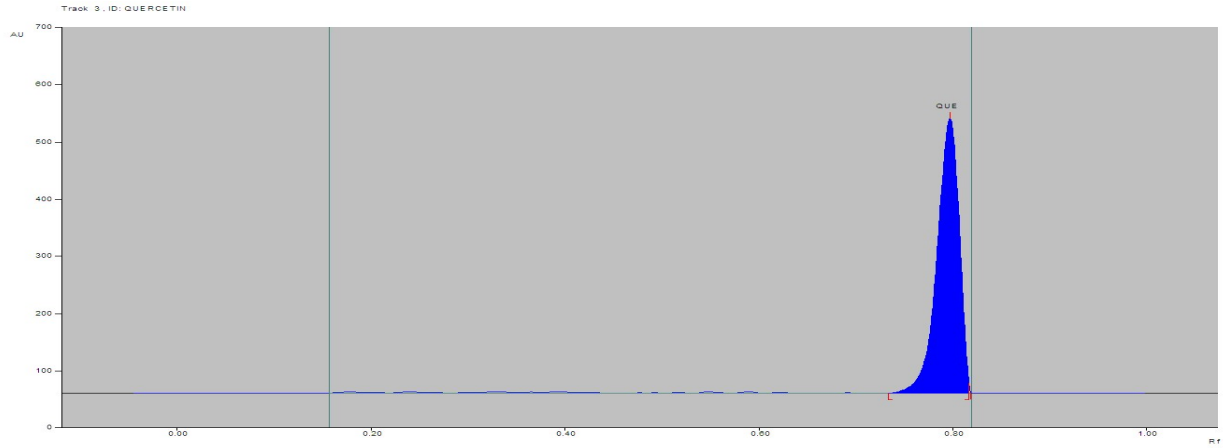

Figure 7: Chromatograms of Standard Quercetin at Rf 7.9

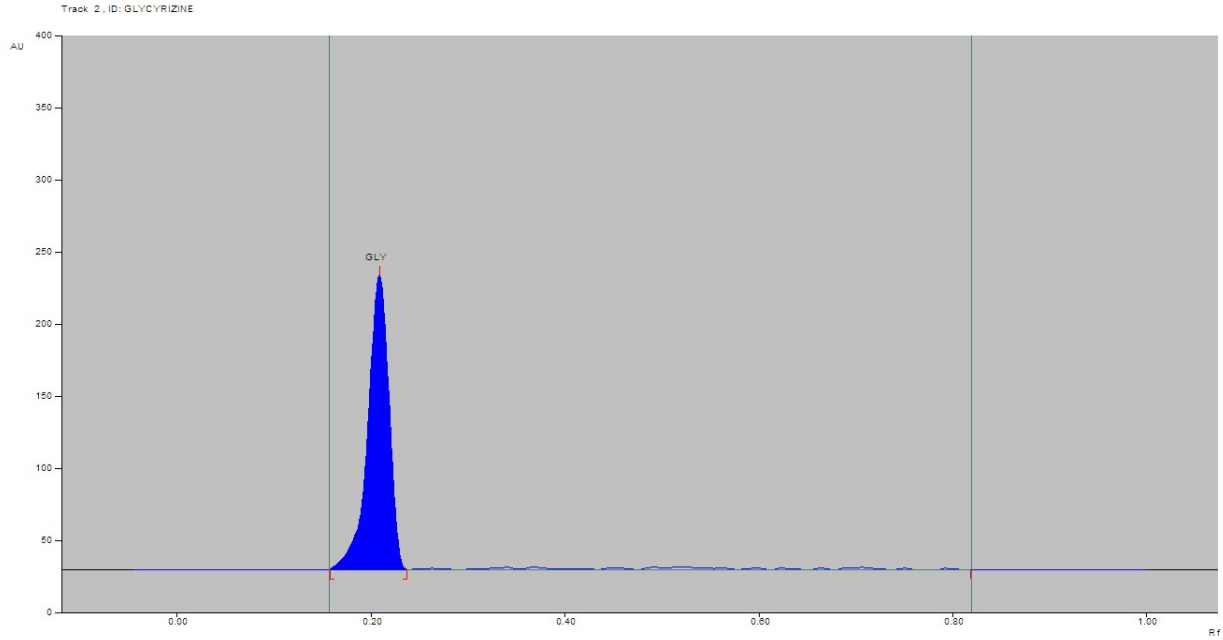

Figure 8: Chromatograms of Standard Glycyrrhizin at Rf 2.2

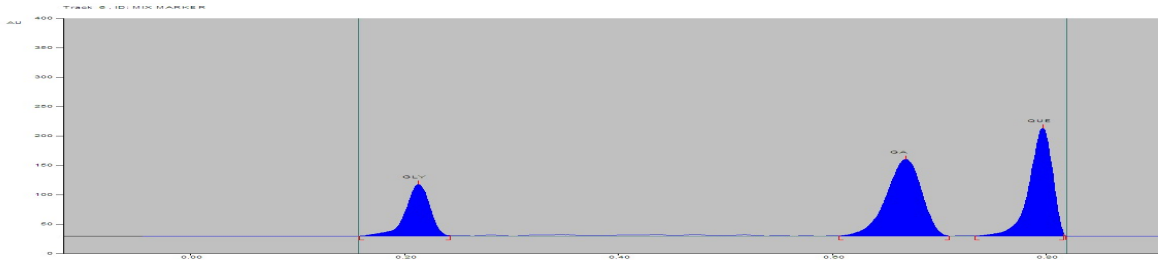

Figure 9: Chromatograms of Mixed Standards (1:1:1)

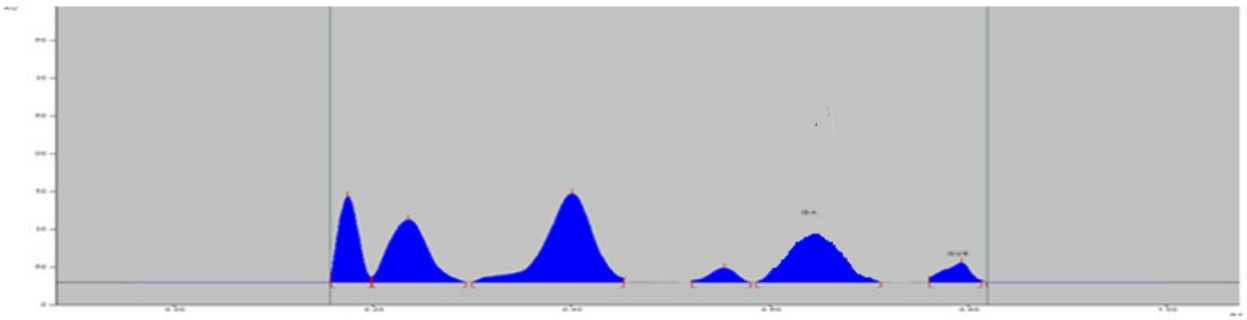

Figure 10: Chromatograms of *methanolic extracts Cordia wallichi* leafs (MECW)

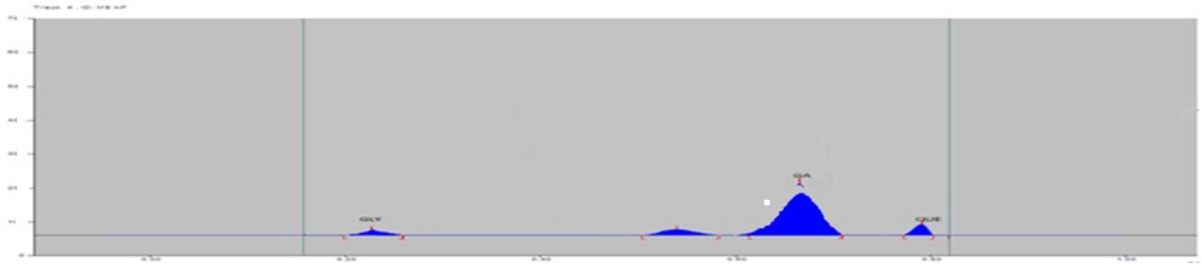

Figure 11: Chromatograms of methanolic extracts of *Abrus precatorius* leaves (MEAP)

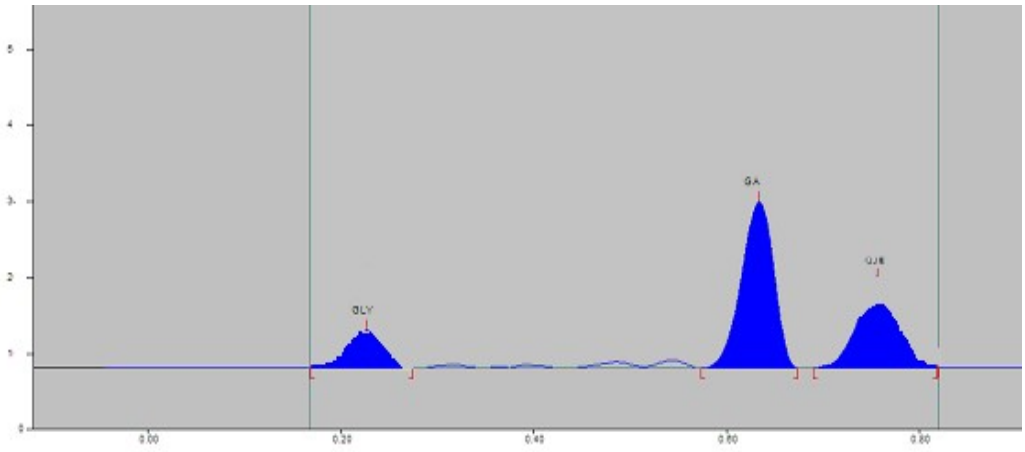

Figure 12: Densitograms of Phytoconstituents in polyherbal tablet (Floating

Table1: Quantitative estimation results of biomarkers in MEAP, MECW and Polyherbal formulation

Methanolic extracts	Peak area of Standard AUC)			Peak Area of the sample (AUC)			% Content		
Abrus precatorius	GAE	QE	GE	GAE	QE	GE	GAE	QE	GE
	9479.5 ±1.0	6973.5 ±5.2	3690.4 ±0.11	11397.2 ±2.0	2225.867 ±0.57	799.56 ±0.57	0.59	0.15	0.11
Cordia wallichi	9479.5 ±1.0	6973.5 ±5.2	3690.4 ±0.11	6779.9 ±10.0	3290.73 ±0.57	ND	0.35	0.23	ND
Polyherbal tablets	13736.9 ±0.01	9579.3 ±0.22	1139.2 ±0.14	320.9 ±0.15	91.63 ±0.24	3.14 ±0.11	0.465	0.192	0.055

Results are expressed in Mean Value± SD; ND: No detected

4. CONCLUSION

Proposed HPTLC method can be used as an analytical tool for quality evaluation of plants and formulations containing as Gallic acid, Quercetin and Glycyrrhizin chemical markers. It is an efficient method to screen *Abrus precatorius* and *Cordia wallichi* samples in order to assess its quality and authenticity. Hence, it can be demonstrated that HPTLC is a powerful practical tool for comprehensive quality control of plant raw materials and it's formulation.

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