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HPTLC fingerprint profile of selected anti-diabetic plants monitored with reference to flavonoids

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ABSTRACT

The main objective of the present study was to evaluate the phytoconstituents and finger print profile in the various extracts of two important anti-diabetic species namely *Terminalia catappa and Cressa cretica.* The plant species were extracted with different solvents such as petroleum ether, chloroform, ethyl acetate and methanol in the increasing order of polarity. Preliminary phytochemical screening of the various extracts of these two species revealed the presence of flavonoids, phenols, tannins and saponins in the methanolic extract. HPTLC methods for the separation of the active constituents in extracts have been developed. HPTLC finger print for different extracts revealed the presence of peaks with R_f values in the range of 0.01 to 0.99. The bands of purple and light yellowish orange colour revealed the presence of flavonoids and phenolic compounds. Routine quality control of these species could be carried out using this hptlc method and it serves in qualitative analysis, quantitative estimation and was appropriate for standardization of the extract.

Keywords: HPTLC, *Terminalia catappa*, *Cressa cretica*, Quercetin, Gallic acid, Rutin.

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The Phytoconstituents of medicinal value have been extensively used for treating various disease conditions. Herbs being easily available to human have been explored to the maximum for their medicinal properties. Products of primary metabolism such as aminoacids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, and terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance. The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with

remarkable bio-activities. Identification and quality evaluation of crude herbal extracts is a fundamental requirement. It is an accepted fact that the qualitative analysis of crude herbal extracts constitutes an important and reliable part of quality control protocol [1]

Standardization and quality control of herbal drugs is very complicated due to group of phytoconstituents and are subjected to variation. Hence, methodologies that can generate a fingerprint of each extract in large collections would be useful to detect stability of the same extract over time. High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters. [2]

Terminalia catappa is a large tropical tree in the lead wood tree family, Combretaceae, that grows mainly in the tropical regions of Asia, Africa, and Australia. It is known by the English common names country-almond, Indian-almond, Malabar-almond, sea-almond, tropical-almond. The tree grows to 35 m (115 ft) tall, with an upright, symmetrical crown and horizontal branches. *Terminalia catappa* has corky, light fruit that are dispersed by water. The seed within the fruit is edible when fully ripe, tasting almost like almond. As the tree gets older, its crown becomes more flattened to form a spreading, vase shape. Its branches are distinctively arranged in tears. The leaves are large, 15–25 cm (5.9–9.8 in) long and 10–14 cm (3.9–5.5 in) broad, ovoid, glossy dark green, and leathery. They are dry-season deciduous, before falling, they turn pinkish-reddish or yellow-brown, due to pigments such as violaxanthin, lutein, and zeaxanthin. The various extracts of the plant have been reported to be anticancer, antioxidant, anti-HIV reverse transcriptase, anti-diabetic, hepatoprotective, anti inflammatory ,anti-hepatitis, and aphrodisiac .The phytochemicals of this plant includes tannins (punicalagin, punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geranin, granatin B, corilagin), flavanoids (isovitexin, vitexin, isoorientin, rutin) [3, 4, 5, 6]

Cressa cretica L. (Convolulaceae), popularly known as 'Rudravanti' in Hindi and is a widely grown halophytic plant. Different parts of the plant have been claimed to be valuable in a wide spectrum of diseases. Traditionally the plant is used in diabetes and asthma. It is used as an expectorant, stomachic, antibilious and altenative. The plant has anthelmintic, stomachic, tonic and aphrodisiac purposes, enriches the blood and is useful in constipation, leprosy, asthma and urinary discharges. It is reported to be antibilious, antitubercular and expectorant. The plant is traditionally used in Bahrain as expectorant and antibilious agent. Dry leaves of C. cretica crushed with sugar are used as emetic in Sudan. [7, 8, 9, 10, 11]

The validation of the novel products in the plants needs powerful analytical devices tailored for the study of herbal extracts in Order to assess composition and face their natural complexity as a resource. The present study is mainly focused to establish the finger print profile of various extracts of *Terminalia catappa* fruit and entire plant of ***Cressa cretica*** using HPTLC. HPTLC based methods could be considered as an important tool in routine drug analysis because of its simplicity and reliability which can be used for identification, authentication.

MATERIALS AND METHODS

Collection and authentication

The entire plant of *Cressa cretica* has been collected from the sandy shores of tuticorin district and authenticated by Dr.C.Kunhikannan, Scientist F and Head of office, Institute of forest genetics and tree breeding, Coimbatore, bearing the Ref.No.940/BD/ID/IFGTB/2017.The fruits of *Terminalia catappa* have been collected from the forest area in the outskirt of Coimbatore and authenticated by Dr.G.V.S. Moorthy, Scientist F and Head of office, Botanical survey of India,coimbatore bearing the Ref.No.BSI/SRC/5/23/2015/Tec/842

Preparation of various extracts

The Fruits of *Terminalia catappa* and the entire plant of *Cressa cretica* was shade dried and powdered coarsely. The coarse powder was refluxed to hot continuous extraction with different solvents such as petroleum ether, chloroform, ethyl acetate and Methanol in the increasing order of polarity using soxhlet apparatus .Extraction was continued until all the constituents are extracted. All the extracts were concentrated and evaporated to dryness. The dried extracts were subjected to preliminary phytochemical analysis and hptlc finger printing profile.

Preliminary phytochemical analysis [12, 13]

All the extracts of *Terminalia catappa* and *Cressa cretica* were subjected to preliminary phytochemical analysis for the presence of various phytoconstituents by using standard methods.

HPTLC fingerprint profile [14, 15]

Preparation of standard solution

Standard solution of gallic acid, rutin and quercetin have been prepared by dissolving 10mg of the sample in methanol and diluted to 10ml (1mg/ml).

Preparation of Extract

5mg/ml concentration of each extracts were prepared in respective solvents of chromatographic grade and then filtered by Whatman filterpaper no.1.

Chromatographic conditions

Chromatogram was developed on 20 x 10 cm aluminium TLC plate precoated with a 0.2 mm layer of silica gel $60F_{254}$ stored in a desiccator. The application was done by Hamilton micro syringe (Switzerland), mounted on a Linomat V applicator. Application of bands of each extract was carried out using spray technique. The sample was applied in duplicate on precoated silica gel $60F_{254}$ aluminum sheets (20 x 10 cm) with the help of Linomat V applicators attached to CAMAG HPTLC system

Optimization of mobile phase

After trials with various mobile phase systems,a composition of Toluene: Ethylacetate: Methanol (5:3:2 v/v) was selected for *Terminalia catappa* and Toluene: Ethylacetate: chloroform: Methanol: Formic acid (2:4.5: 1.5:1:1 v/v) for *cressa cretica* The spotting was done on the TLC plate and developed by ascending development technique with in a camag chamber previously saturated with respective mobile phase for 30 mins. After development, the plate was dried and subjected to scanning.

Development of Chromatogram

Densitometric scanning of the plate was then performed with a Camag TLC Scanner 3 equipped with the win CATS Software. The chromatograms were scanned by the densitometer at 254 and 366 nm for the extracts of *Cressa cretica* and 312nm for *Terminalia catappa* fruit.

RESULT AND DISCUSSION

Preliminary phytochemical screening

The Phytochemical test on various extracts of *Terminalia catappa* fruit showed the presence of various phytoconstituents like Terpenoids, steroids, saponins, flavonoids, phenols and tannins in the methanolic extract extract (Table 1).

The Phytochemical test on various extracts of entire plant of cressa cretica showed the presence of various phytoconstituents like Terpenoids,steroids, flavonoids, phenols and tannins in the methanolic extract (Table 2).

HPTLC profile

Terminalia catappa fruit extract

Different mobile phases were examined in order to achieve high resolution and reproducible peaks. The study revealed that Terminalia catappa showed best results in Toluene: Ethyl Acetate: Methanol (5:3:2) solvent system for all the extracts. After scanning and visualizing the plates in absorbance mode at both 254nm, 366 nm best results were shown at 254nm. The HPTLC images shown in fig. 1-4 indicate that all sample constituents were clearly separated. The results for HPTLC finger print was scanned at wavelength 254 nm for all the extracts. Methanolic extract of *Terminalia catappa* revealed the presence of many phytoconstituents .The Rf values ranged from 0.03 to 0.99.The component peak with Rf value 0.06 and 0.61 matches with the peak and also spectra of Rutin and gallic acid respectively. TLC plate showed different colour phytoconstituents of Terminalia

catappa methanolic extract .The bands revealed presence of purple, and yellowish orange bands showing the presence of Flavanoids and phenolic compounds. Mobile phase with- Toluene: Ethyl Acetate: Methanol (5:3:2) showed high resolution and reproducible peaks.

Cressa cretica extract

The study revealed that ***Cressa cretica*** extractshowed best results in Toluene: Ethylacetate: chloroform: Methanol: Formic acid (2:4.5: 1.5:1:1) solvent system for all the extracts. After scanning and visualizing the plates in absorbance mode at 312 nm best results were obtained. The HPTLC images shown in fig.5-8 indicate that all sample constituents were clearly separated. Methanolic extract of *Cressa cretica* revealed presence of many phytoconstituents with the Rf values ranging from 0.03 to 0.99.The component peak with Rf value 0.03 and 0.51 matches with the peak and also spectra of rutin and quercetin respectively. TLC plate showed purple, and yellowish orange bands in the methanolic extract of Cressa cretica. These bands revealed the presence of flavanoids.

Table. 1 Preliminary phytochemical analysis of *Terminalia catappa*

Phyto constituents	Pet. Ether	chloroform	Ethylacetate	Methanol
CARBOHYDRATES				
1. Molisch's Test	-	+	+	++
2. Fehling's test	-	+	+	++
3. Benedict's test	-	+	+	++
PROTEIN				
1. Biuret's test	-	-	-	-
2. Millon's test	-	-	-	-
3. Precipitation test	-	-	-	-
AMINO ACIDS				
1. Ninhydrin test	-	-	-	-
FATS AND OILS				
1. Solubility	-	-	-	-
2. Saponification	-	-	-	-
STEROID				
1. Salkowski reaction	-	+	-	-
LIEBERMANN-BURCHARD REACTION				
1. Liebermann's reaction	-	-	+	+
GLYCOSIDES				
1. Baljet's test	-	-	-	-
2. Legal's test	+	+	+	-
3. Borntrager's test	-	-	-	-
SAPONIN GLYCOSIDES				
1. Foam test	-	-	-	+
2. Haemolytic test	-	-	-	-
FLAVONOIDS				
1. Shinoda test	-	-	-	+++
ALKALOIDS				
1. Dragendorff's test	-	-	-	-
2. Mayer's test	-	-	-	-
3. Hager's test	-	-	-	-
4. Wagner's test	-	-	-	-

UNSATURATED HYDROCARBONS				
Bromine test				
KMnO4 test	-	-	-	-
	-	-	-	-
TERPENOID				
1. Liebermann'sBuchard reaction	-	-	-	+
2. Hirschorn test				
	-	-	-	-
TANNINS AND PHENOLIC COMPOUNDS				
1. 5% FeCl3				
2. Lead Acetate	-	-	-	+++
3. Gelatin	-	-	-	+++
	-	-	-	+++

Table. 2 Preliminary phytochemical analysis of *Cressa cretica*

Phyto constituents	**Pet. Ether**	**chloroform**	**Ethylacetate**	**Methanol**
CARBOHYDRATES				
1. Molisch's Test	-	-	-	+
2. Fehling's test	-	-	-	+
3. Benedict's test	-	-	-	+
PROTEIN				
1. Biuret's test	-	-	-	-
2. Millon's test	-	-	-	-
3. Precipitation test	-	-	-	-
AMINO ACIDS				
1. Ninhydrin test	-	-	-	-
FATS AND OILS				
1. Solubility	-	-	-	-
2. Saponification	-	-		
STEROID				
1. Salkowski reaction	-	-	-	-
LIEBERMANN-BURCHARD REACTION				
1. Liebermann's reaction				
	-	-	-	-
GLYCOSIDES				
1. Baljet's test	-	-	-	-
2. Legal's test	-	-	-	+
3. Borntrager's test	-	-	-	-
SAPONIN GLYCOSIDES				
1. Foam test	-	-	-	-
2. Haemolytic test	-	-	-	-
FLAVONOIDS				
1. Shinoda test	-	-	++	+++
ALKALOIDS				
1. Dragendorff's test	-	-	-	-
2. Mayer's test	-	-	-	-
3. Hager's test	-	-	-	-
4. Wagner's test	-	-	-	-

UNSATURATED HYDROCARBONS				
1. Bromine test	-	-	-	-
2. KMnO4 test	-	-	-	-
TERPENOID				
1. Liebermann'sBuchard reaction	-	-	-	+
2. Hirschorn test	-	-	-	-
TANNINS AND PHENOLIC COMPOUNDS				
1. 5% FeCl3				
2. Lead Acetate	-	-	-	+++
3. Gelatin	-	-	-	+++
	-	-	-	+++

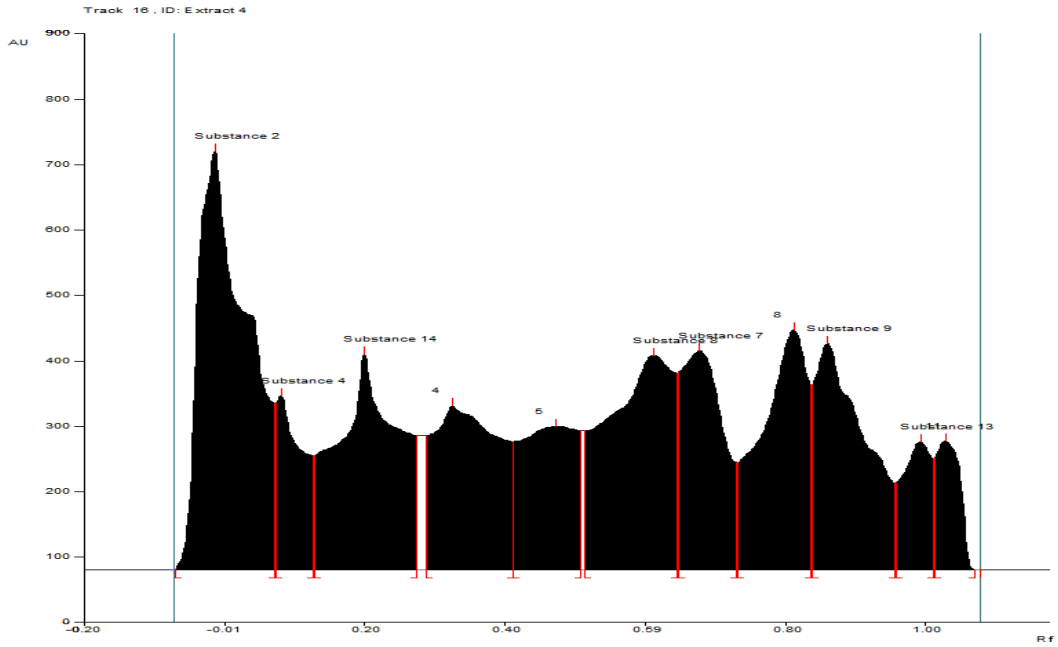

Fig.1 Finger print of Alcoholic extract of *Terminalia catappa*

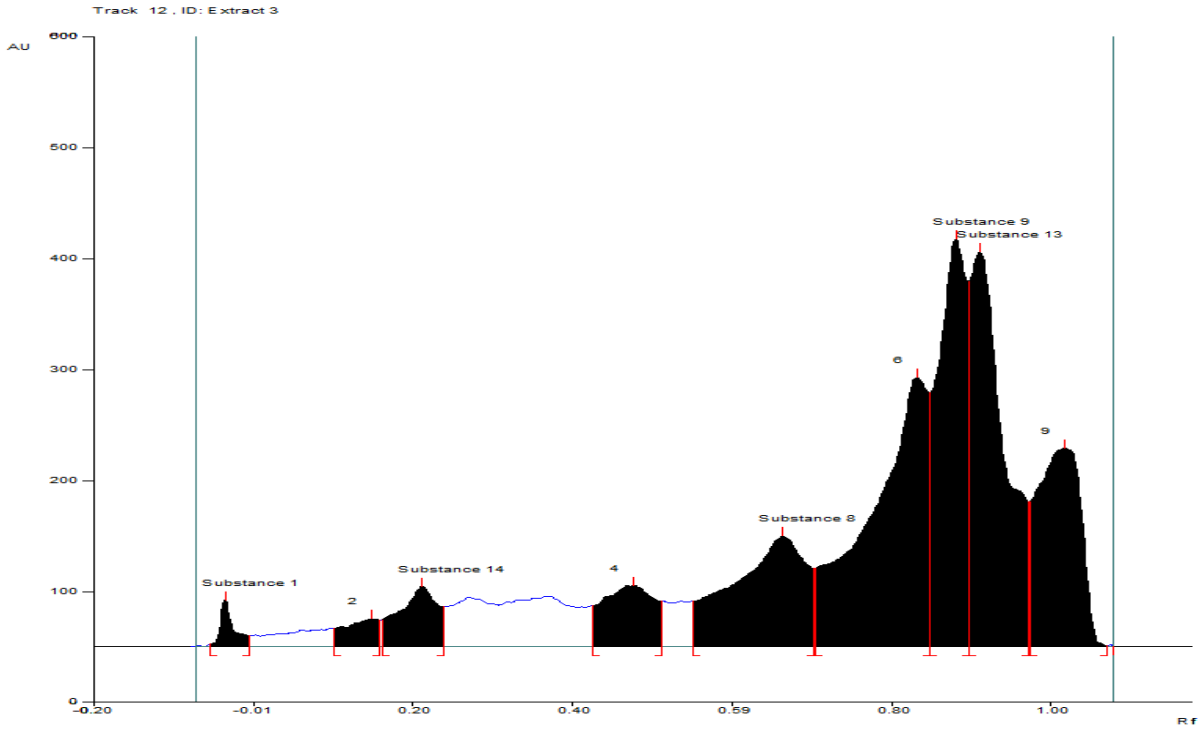

Fig.2 Finger print of Ethyl acetate extract of *Terminalia catappa*

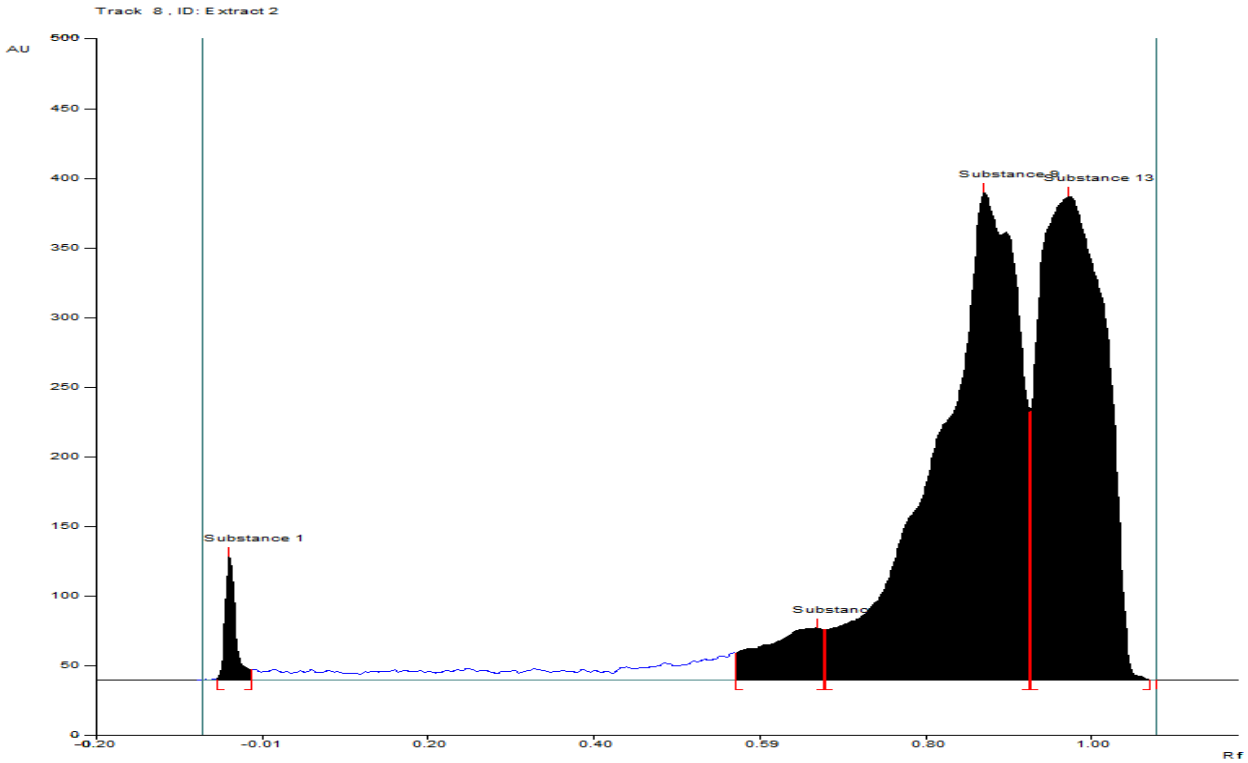

Fig.3 Finger print of chloroform extract of *Terminalia catappa*

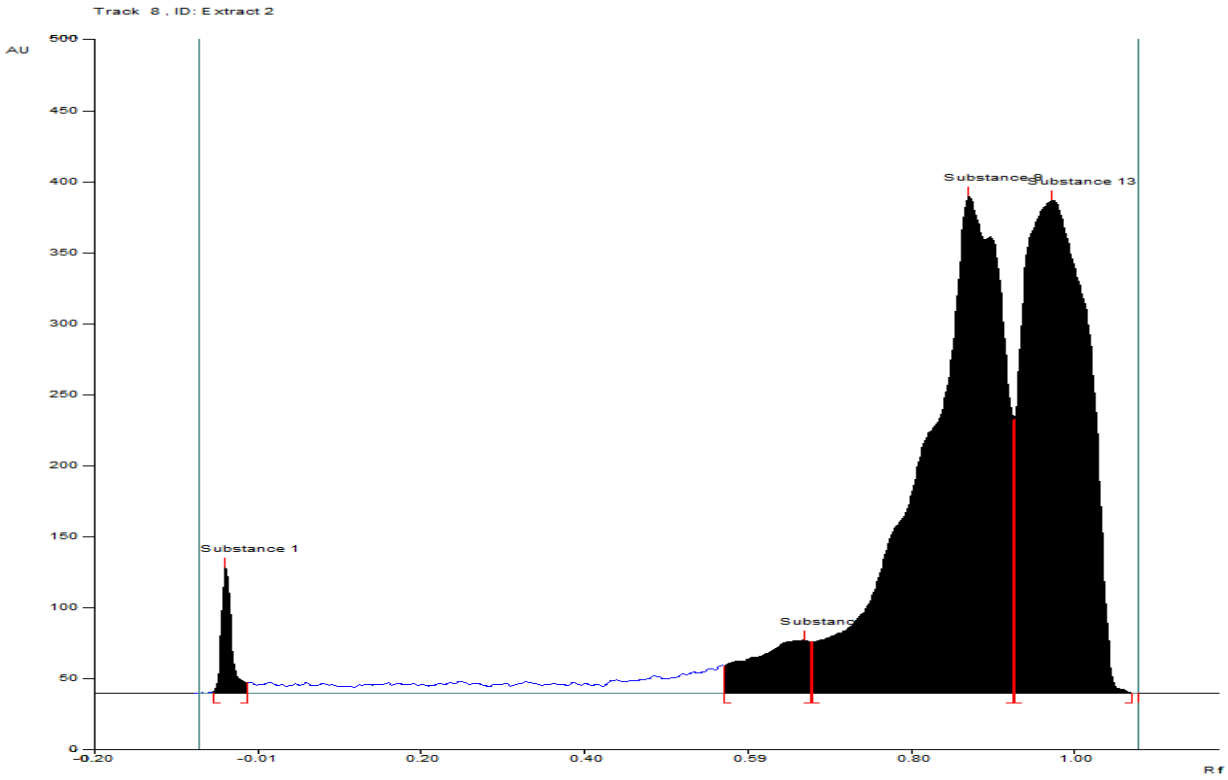

Fig.4 Finger print of petroleum ether extract of *Terminalia catappa*

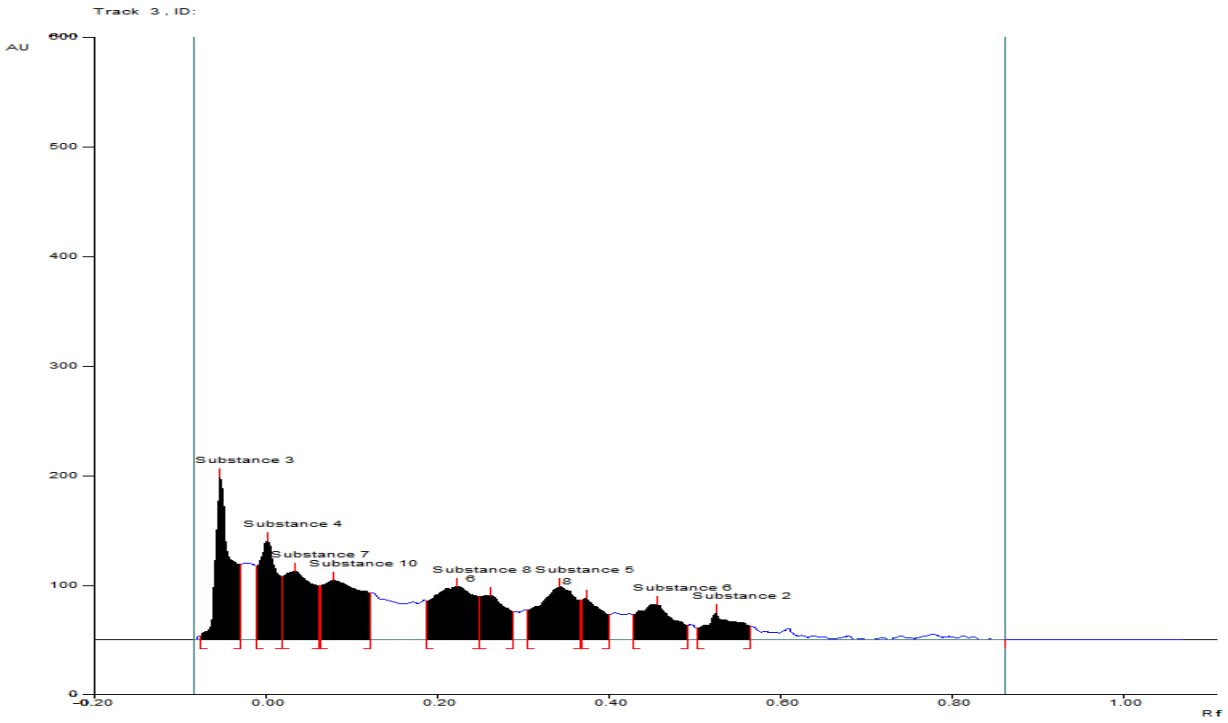

Fig.5. Finger print of Alcoholic extract of *Cressa cretica*

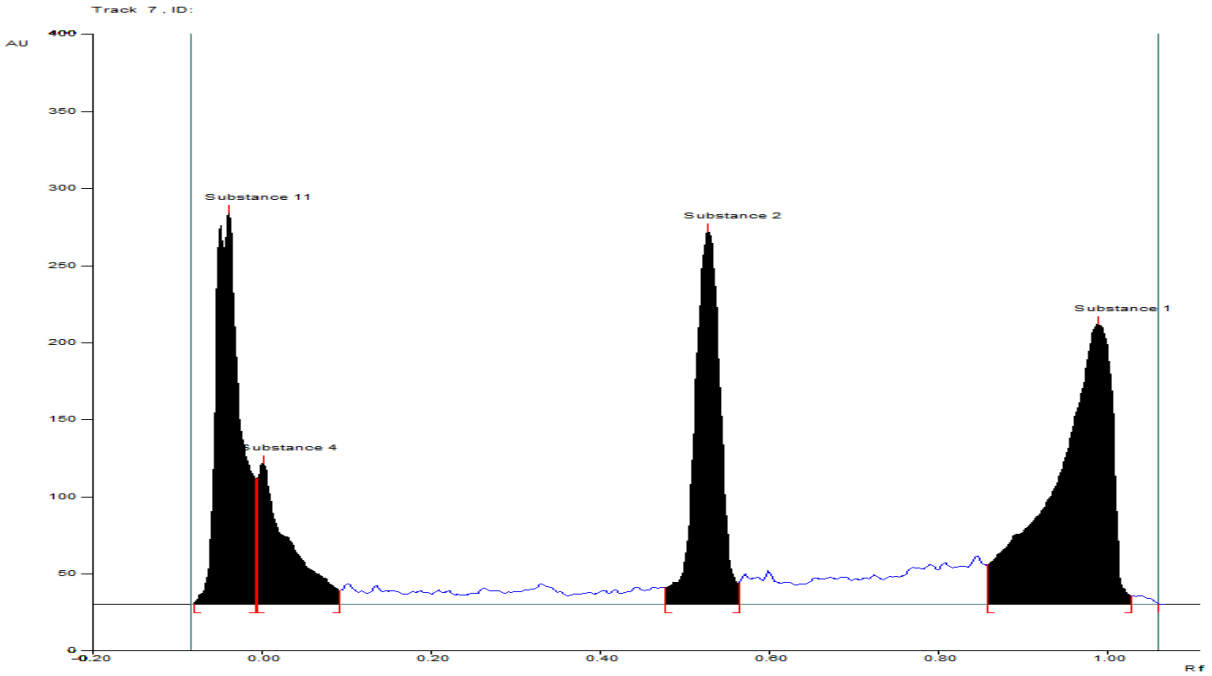

Fig.6 Finger print of ethyl acetate extract of *Cressa cretica*

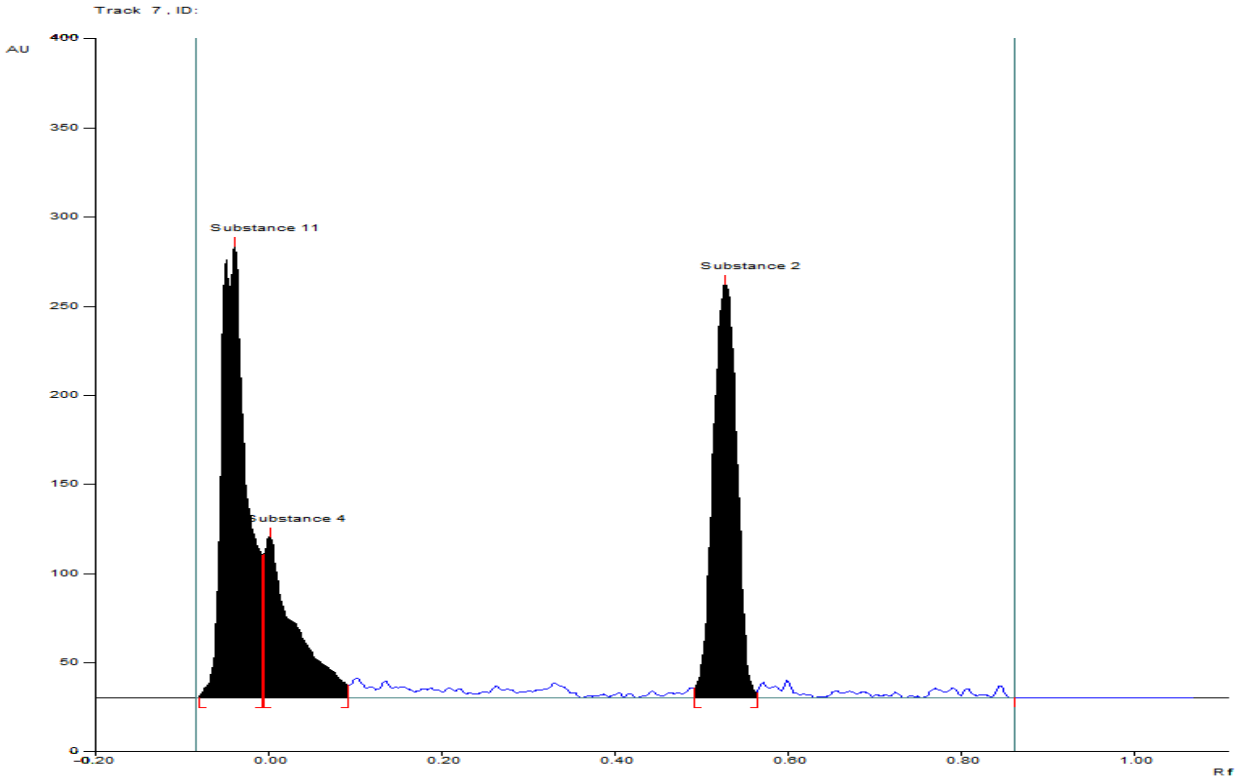

Fig.7 Finger print of chloroform extract of *Cressa cretica*

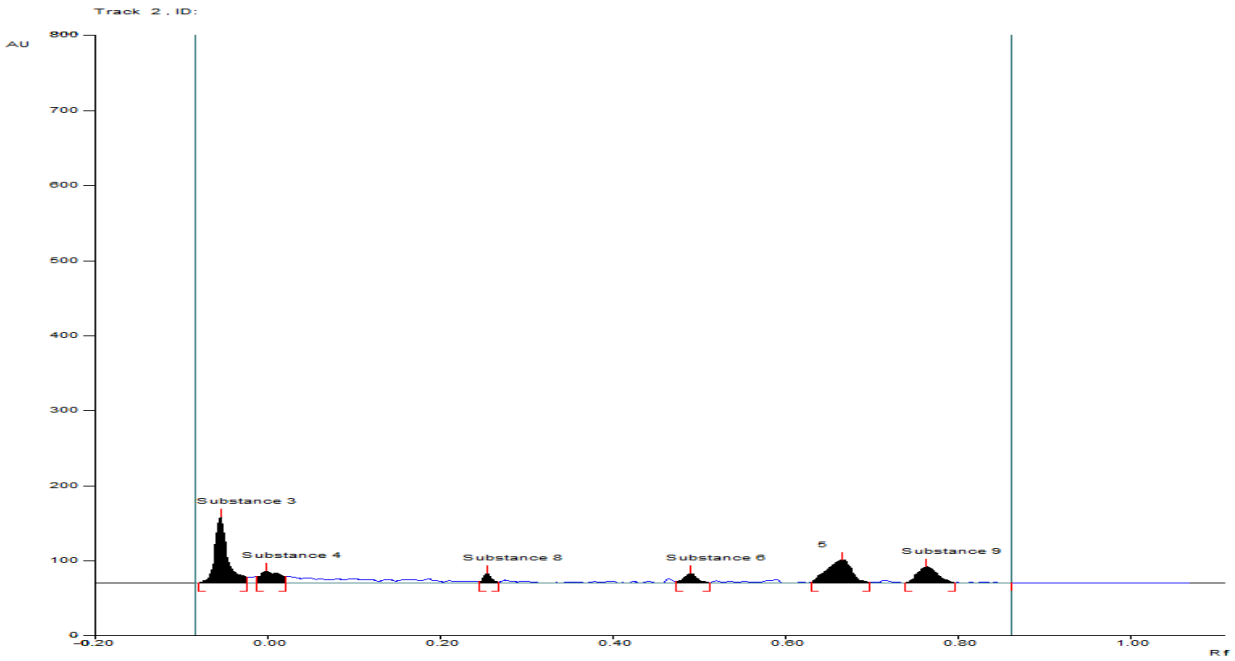

Fig 8 Finger print of petroleum ether extract of *Cressa cretica*

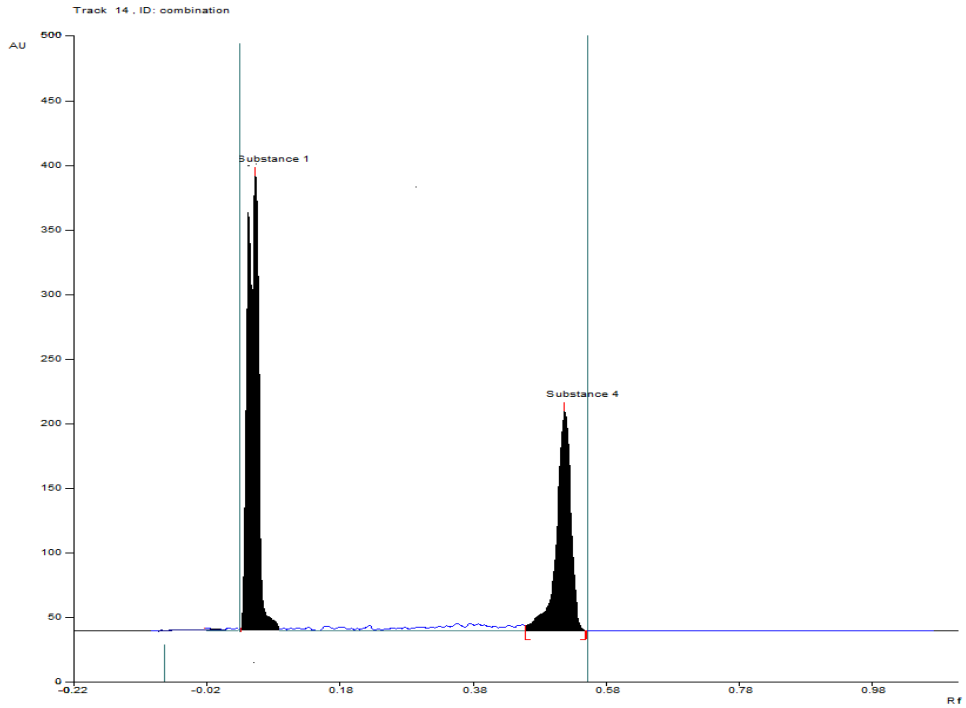

Fig 9. Rutin and quercetin

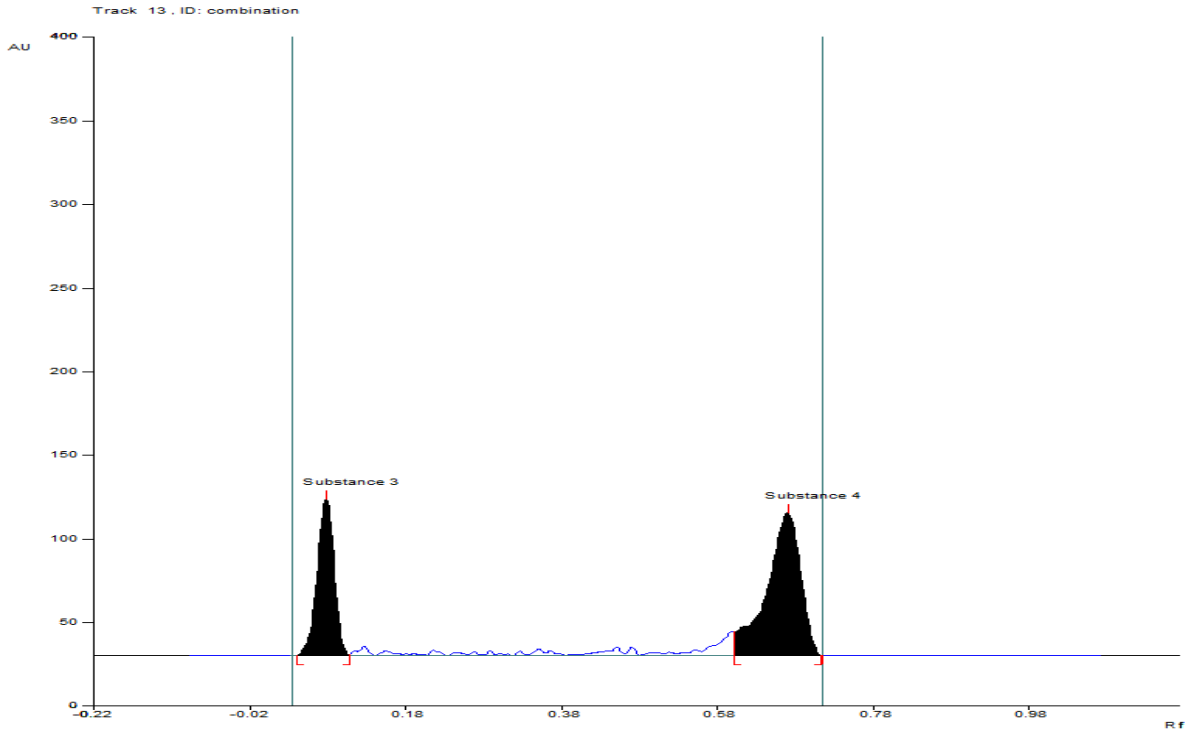

Fig 10. Rutin and gallic acid

CONCLUSION

The Phyto constituents are the natural bioactive compounds found in plants. The medicinal plants contain many of the phytoconstituents in which the phenolic compounds and flavanoids could be useful for many therapeutic purposes as they often contain huge amount of medicinal properties. The results in the work demonstrates that the extracts of selected medicinal plants contain considerable quantity of phenolic compounds and flavonoids. This newly developed HPTLC method helps to estimate major plant flavonoids. The HPLC analysis confirmed the presence of quercetin and rutin in the ethanolic extracts of *cressa cretica*, also the presence of rutin and gallic acid in the ethanolic extract of *Terminalia catappa*. The results of the present study directly coincided with the previous observations. The developed HPTLC method will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization, and quality control of studied species.

Thus the developed chromatogram is specific with selected mobile phase system and serves the better tool for standardization of the extract. HPTLC fingerprint of a plant species helps in the proper identification and quality control of a particular plant species and also provides basic information regarding isolation, purification, characterization and identification of marker chemical compounds of the species. Thus, the present study provides sufficient information about phytoconstituents present in the different extracts of ***Terminalia catappa and Cressa cretica***. Further investigation on the isolation, purification and characterization of the phytoconstituents in these plants may reveal the potentiality of these compounds in pharmaceutical industry.

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