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Analyzing Phenols and Triterpenoids in Dechaschistia Crotonifolia Leaves Using HPTLC Fingerprinting

Raveesha Peeriga[1*], Aminabee Shaik*, Manasa Gude[1], Tejaswini Sonti[1], Mounika Cheboina[1], Bhagya Rekha Penumala[1], Ganesh KVB[2]

[1]V. V. Institute of Pharmaceutical Sciences, Seshadri Rao Knowledge village, Gudlavalleru – 521356, Krishna District, Andhra Pradesh, India.

[2]KL College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh, India.

Abstract

Utilizing HPTLC fingerprint analysis, it is simple to analyze and understand an extract made up of various phytoconstituents from a qualitative perspective. Dechaschistia crotonifolia (Burn. f.) has been employed in traditional medicine systems for a very long time. Given that HPTLC is a very reliable method of analysis, it was chosen to interpret the phytoconstituents, namely phenols and triterpenoids, in Dechaschistia crotonifolia (Burn. f.), in accordance with Current Good Manufacturing Practices, which emphasize the importance of quality in regard to phytoconstituents. This study uses the CAMAG HPTLC System connected to the Linomat 5 Applicator, TLC Visualizer, and Scanner to create an HPTLC finger print profile for the secondary metabolites, specifically phenols and triterpenoids, in the leaf methanolic extract of Dechaschistia crotonifolia (Burn. f.). Numerous peaks were visible in the chromatogram when phytoconstituents were evaluated using HPTLC densitometric analysis at wavelengths of 254 nm and 366 nm. The phytochemicals are evaluated by interpreting the peak areas, peak heights, and Rf values that were reported in the appropriate tables. Triterpenoids, which are phytoconstituents, were found in the study. When the Rf values of these compounds are compared with standards as a reference, this information is very helpful in exploring chemical profiling and identifying bioactive ingredients.

Keywords: Phenols, Triterpenoids, Metabolites, HPTLC-fingerprints, Chromatogram.

Introduction

The significance of botanical identity is required by Current Good Manufacturing Practices (cGMPs)[1]. Measuring chemical variability and precisely identifying components are necessary for quality assurance of herbal remedies. By using an analytical technique, it was possible to identify species variation at numerous phytoconstituents within the genus [2]. The most advanced analytical technique currently available, high-performance thin-layer chromatography (HPTLC), has numerous uses in the identification of medicinal plants, the detection of adulterations, and the quantification of phytoconstituents [3]. The validity of the phytoconstituents will guarantee the medicinal potential of the plants used in Indian traditional medicines. HPTLC fingerprinting analysis is the qualitative method used to evaluate the efficacy and function of herbs [4].

In the deciduous forests of central India, *Dechaschistia crotonifolia Wight & Arn.* (Ebaenaceae) is a plant that is frequently grown. Given that it is a 10 celled locucidal capsule genus, the name Dechaschistia is derived from the Greek words "deka" for ten and "schistos" for cleft [5–6]. In the current study, a methanolic extract of Dechaschistia crotonifolia leaves was employed to perform a phytochemical profile using HPTLC as an analytical method.

Methods

Authentication

Prof. K. Madhava Shetty, Department of Botany, SV University, Tirupati, Andhra Pradesh, India, identified the plant material.

Extraction

The leaves were manually plucked from Gudlavalleru, washed, and sun-dried for seven days. The dehydrated leaves were mechanically ground. Using a Soxhlet device, 100g of crushed leaves had been extracted with methanol before being concentrated with a rotary evaporator.

Instrumentation

The CAMAG HPTLC system consisted with an ATS 4 applicator, a TLC visualizer, a TLC scanner 4, and Lab Server Software.

Solvents and chemicals

All of the substances and solvents used in the inquiry were present in chromatographically graded samples.

HPTLC Specifications

Preparation of Sample

1 mg of methanolic *Dechaschistia crotonifolia* extract of leaves was produced, concentrated in 1 ml of a methanol and filtered.

Development Chamber

After applying the sample, the HPTLC plate was held in a development chamber with mobile phase. For triterpenoids and phenols chloroform: methanol. Ice-bound acetic acid Water (64:32:12:8) were used.

Derivatization

For phenols the developed plate is sprayed with Alcoholic $FeCl_3$. Alcoholic $FeCl_3$ reagent is prepared by dissolving 2g $FeCl_3$ is dissolved in 10mL water and diluted to 200mL with ethanol heated 100°C for 3 min and for triterpenoids the developed plate is sprayed with Anisaldephyde Sulphuric acid reagent [7-8].

Visualization

With the aid of TLC Scanner 4, the generated bands were scanned at wavelengths of 254 nm and 366 nm with an optimal resolution.

Results and Discussion

By looking at the formed peaks in each chromatogram, the HPTLC analysis of the methanolic leaf extract of Dechaschistia crotonifolia revealed the presence of phenols and triterpenoids (Figs. 2 to 7). The chromatograms were recorded at 254 nm and 366 nm wavelengths. The peak areas, heights, Rf values, and percent areas of the phytochemicals are displayed in the tables of chromatograms, and each chromatogram is detailed in the discussion section. (Tab. 1 to 6). The HPTLC fingerprint analysis of the methanolic leaf extract of Dechaschistia crotonifolia revealed the presence of triterpenoids and the absence of phenols. Figure 1 displays the produced band on the HPTLC plate that was analyzed about 254nm and 366nm.

The chromatograms obtained for the finger printing examination of phenols (Fig. 2, 3 & Tab. 1, 2) revealed two peaks with Rf values of 0.092 and 0.192, signifying the presence of two different types of phenols, and peak heights (H) of 0.0221 and 0.0240 scanned at 366 nm. The peak at 254 nm was not well distinguished.

At 254 nm, triterpenoids produced one peak with an Rf value of 0.689 and a peak height (H) of 0.0292. After derivatization, there were ten peaks at 366 nm with Rf values of 0.048, 0.103, 0.168, 0.334, 0.495, 0.561, 0.666,

0.723, 0.797, and 0.860 and peak heights (H) of 00.103, 0.0125, 0.0329, 0.0131, 0.0807, 0.1804, 0.1159, 0.1284, 0.1203, and 0.1075.

These Rf values indicate that tritepenoids exist but not phenols. It is simpler to identify the kind and quantity of botanical elements present in the plant using the Rf and peak values computed by HPTLC. On the HPTLC plates, which were rendered visible under UV of wavelengths 254 nm and 366 nm, respectively, the separated compounds were clearly apparent. (Fig. 1). Plant-derived phytoconstituents are crucial for the manufacturing of pharmaceuticals since they have been used for treating both acute and chronic disorders since the beginning of time [9–10]. For the study and development of phytomedicines, a rapid analytical method is required due to the need for plants [11–12].

The study of phytochemicals and biological molecules, the measurement of pharmaceuticals and active ingredients, formulation fingerprinting, and the detection of adulterants in formulations are all examples of applications for HPTLC. Chemicals of forensic importance can be located using HPTLC. The use of a hyphen in HPTLC-MS, HPTLC-FTIR, and HPTLC-Scanning Diode Laser, in addition to other cutting-edge HPTLC-related techniques, has made HPTLC a powerful analytical tool. The analysis of drug formulations, bulk pharmaceuticals, and natural materials will benefit more from the application of HPTLC in the future, according to experts[13]. Thus, the main objective of this research is to identify the botanical elements, such as glycosides, essential oils, and tannins, using HPTLC finger print analysis.

As UV filters, attractants, structural polymers (like lignin), signaling components (like salicylic acid and flavonoids), defense response chemicals, and antioxidants, plant phenolic compounds can serve a variety of purposes. (Phytoalexins, tannins). From a human physiological standpoint, phenolic compounds play a significant part in defense reactions such wrinkle reduction, anti-inflammatory, antioxidant, and anti-proliferative properties. It is desirable to eat plant foods with high antioxidant chemical content in order to lower the chance of acquiring certain chronic diseases, such as diabetes, cancer, and cardiovascular disorders. This is accomplished by controlling oxidative stress. As UV filters, attractants, structural polymers (like lignin), signaling components (like salicylic acid and flavonoids), defense response chemicals, and antioxidants, plant phenolic compounds can serve a variety of purposes. (Phytoalexins, tannins). From a human physiological standpoint, phenolic compounds play a significant part in defense reactions such wrinkle reduction, anti-inflammatory, antioxidant, and anti-proliferative properties. It is desirable to eat plant foods with high antioxidant chemical content in order to lower the chance of acquiring certain chronic diseases, such as diabetes, cancer, and cardiovascular disorders. By controlling oxidative stress, this is accomplished[14–15].

The primary sources of bioactive molecules are natural items, and they have also been crucial in finding the key components for creating drugs to treat human disorders [16]. Isoprenoids, biflavanoids, and alkaloids are three secondary metabolites with bioactive potential that are found in medicinal plants and are particularly important. The entire manufacturing of anticancer chemicals has been prompted by the discovery of anticancer molecules [17–19].

The enormous metabolic potential of every living organism, especially plants, can only be fully explained by terpenoids, which are secondary metabolites [20–22]]. The most fundamental branched C5 unit, isoprene (2-methyl-1,3-butadiene), is assumed to be the source of all terpenoids. In general, a chemical is referred to as a terpene if it has an absolute number of C5 units. While terpenes are generally thought of as basic hydrocarbons, terpenoids are a structurally changed category of terpenes with different types of functional groups and oxidized methyl groups relocated or deleted at various sites [23–27]. dependent on how many C5 units are present in the molecule Terpenoids are classified into monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes based on their isoprenoid C5 units.

Most of the terpenoids that were isolated from medicinal plant products showed immunobiologic properties, and they can be employed all over the world, especially for the treatment of various infectious diseases. Anticancer drugs like Taxol and its derivatives are made using some terpenoids [28–30]. Terpenes are a key ingredient in many artificial flavors and attractive scents because of their pleasing aroma. On the other hand, terpenoids, such as artemisinin and similar substances, are also used as antimalarial drugs. They also play a number of different roles in the industries of vitamins, hormones, food, and cosmetics [31, 32].

The Dechaschistia crotonifolia leaf included a variety of terpenoids, according to the study's findings. The current study thus supports the fact that traditional medicine is successful in treating a wide range of illnesses. Many plants contain secondary metabolites, including alkaloids, flavanoids, saponins, terpenes, and others, that are used as medicinal agents as well as in the pesticide and cosmetic industries. The authenticity of medicinal plants in terms of both genetic and chemical characteristics is a critical need for the use of these botanicals in research. In the era of molecular biology, taxonomy and the morphological traits of plants are helpful in the systematic study of plants and their classification. The classification is also based on biochemical, anatomical, cytological, and molecular characteristics. The HPTLC profile (Chemical profile) of the methanolic extracts of *Dechaschistia crotonifolia* complements, improves, and confirms the study's HPLTC profile's identification of phenols and triterpenoids. The understanding of the chemical components that are present aids in the chemo-taxonomical classification of the plant.

Triterpenoids are present while phenols are absent when scanned at wavelengths of 254 nm and 366 nm, respectively. Ten distinct forms of triterpenoids were found, according to the findings of the current study. Glycosides, essential oils, and tannins have different biochemical levels that can be seen in the HPTLC profiles of these substances. The plant's medicinal significance can then be further defined and authenticated using a linear, precise, and accurate HPTLC fingerprinting approach.

In order to determine the presence of various plant-based constituents like phenols and triterpenoids from chromatogram peaks and acquire the peak tables, the current study's findings are restricted to the HPTLC examination of a methanolic extract of leaves from Dechaschistia crotonifolia. However, the study still needs to take quantitative measurements of these phytoconstituents.

Conclusion

The Dechaschistia crotonifolia plant reportedly contains triterpenoids and lacks phenols, which are utilized in conventional medicine to treat a variety of diseases. Isolated chemical entities and their profile are crucial for the creation of medicines. When the Rf values of these compounds are compared with standards as a guide, the HPTLC analysis for methanolic leaf extract of Dechaschistia crotonifolia is useful in chemical profiling and identifying bioactive components.

Acknowledgements

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Conflict of interests:

The authors declared no conflicts of interest.

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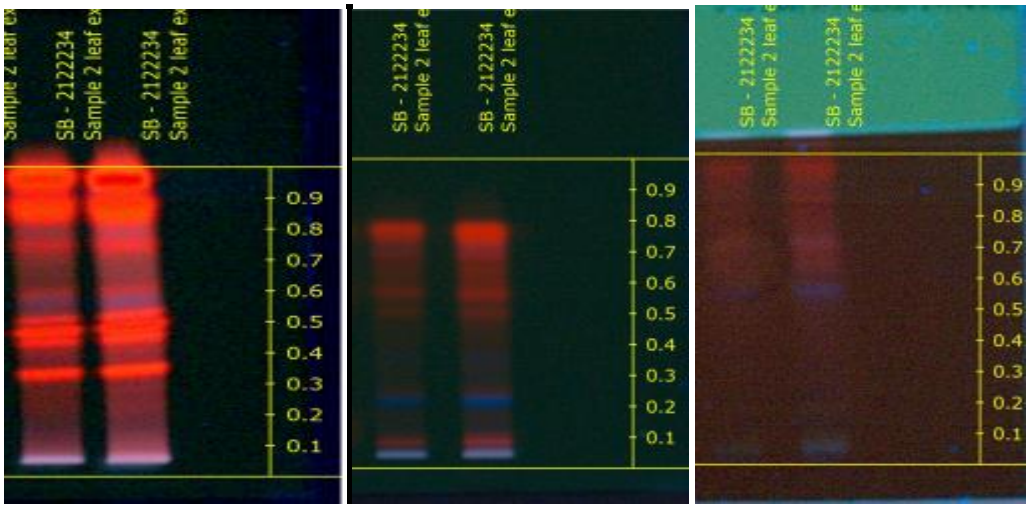

Figure 1: HPTLC plates A: Flavonoids, B: Terpenoids exhibiting developed bands where as C: Phenols not exhibiting well defined bands

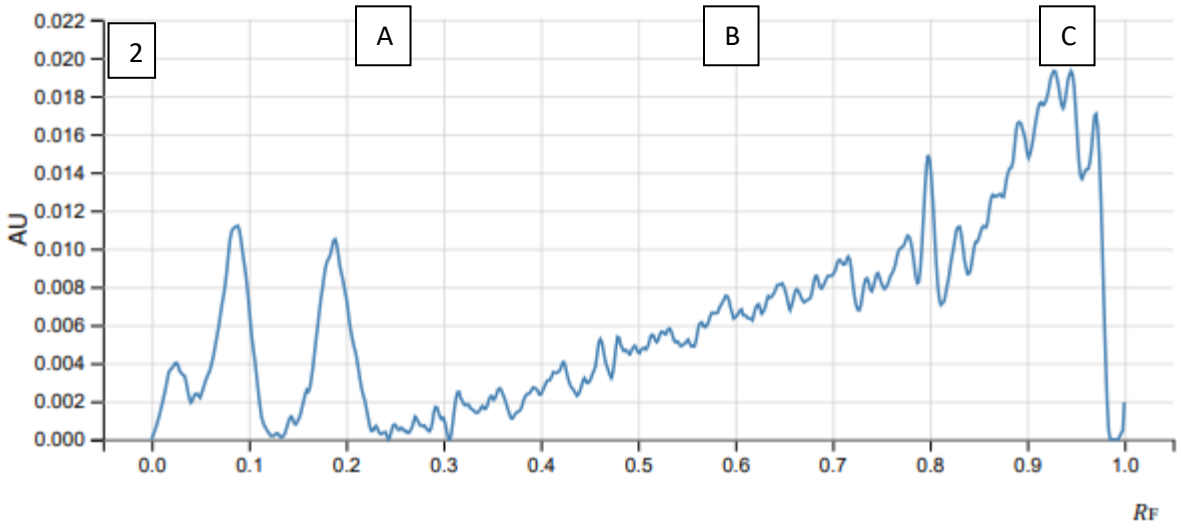

Figure 2: HPTLC finger print analysis for phenols after derivatization scanned at 264 nm

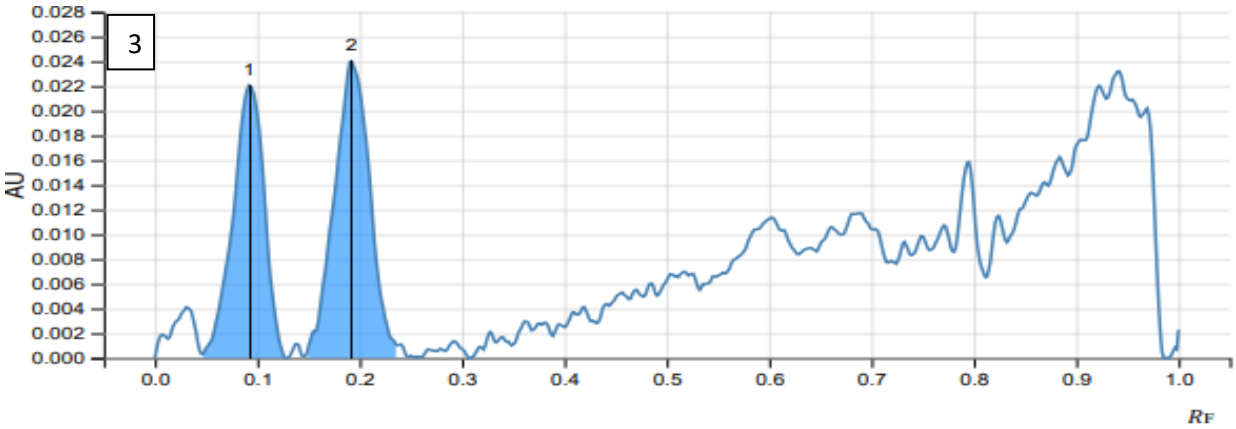

Figure 3: HPTLC finger print analysis for phenols after derivatization scanned at 366 nm

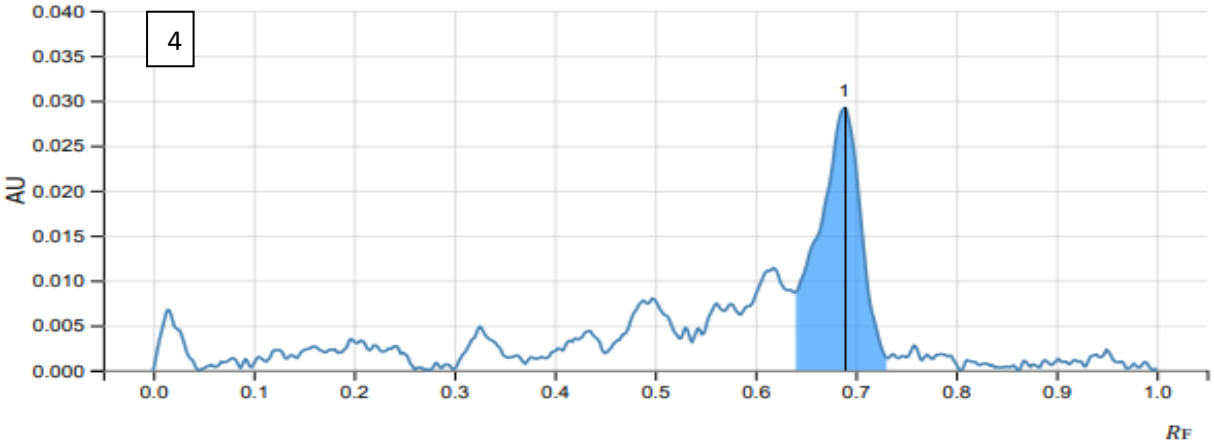

Figure 4: HPTLC finger print analysis for triterpenoids after derivatization scanned at 264 nm

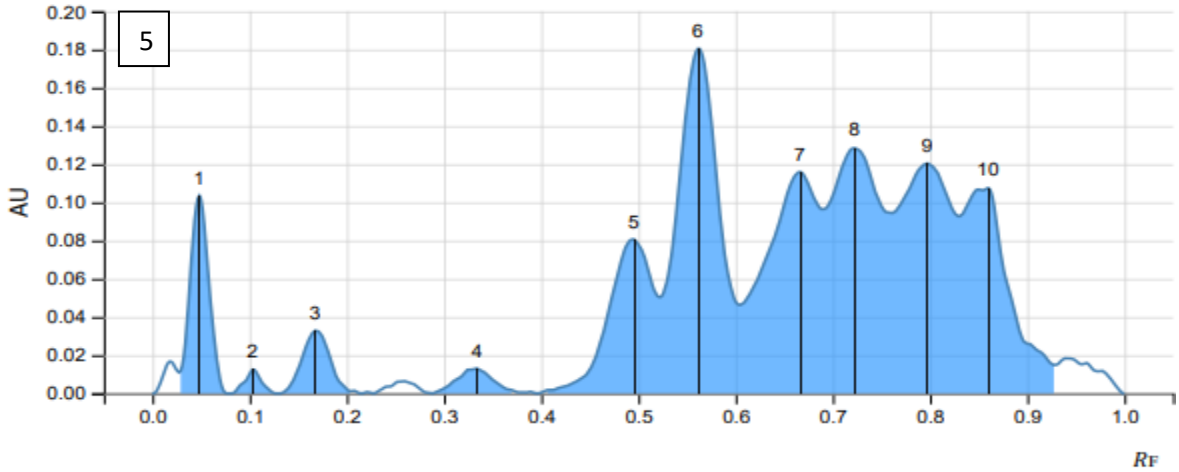

Figure 5: HPTLC finger print analysis for triterpenoids after derivatization scanned at 366 nm

Table 1: HPTLC finger print analysis for flavonoids showing R_f values and Peak Height (H) at 366 nm

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.047	0.0003	0.092	0.0221	47.89	0.127	0.0000	0.00079	44.77	No
2	0.148	0.0004	0.192	0.0240	52.11	0.237	0.0010	0.00097	55.23	No

Table 2: HPTLC finger print analysis for triterpenoids showing R_f values and Peak Height (H) at 254 nm

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.639	0.0086	0.689	0.0292	100.00	0.732	0.0013	0.00145	100.00	No

Table 3: HPTLC finger print analysis for triterpenoids showing R_f values and Peak Height (H) at 366 nm

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.027	0.0110	0.048	0.1034	11.55	0.076	0.0000	0.00234	5.12	No
2	0.082	0.0000	0.103	0.0125	1.39	0.127	0.0000	0.00024	0.52	No
3	0.132	0.0000	0.168	0.0329	3.67	0.203	0.0011	0.00108	2.37	No
4	0.287	0.0000	0.334	0.0131	1.46	0.376	0.0006	0.00056	1.22	No
5	0.402	0.0009	0.495	0.0807	9.02	0.521	0.0505	0.00417	9.11	No
6	0.523	0.0504	0.561	0.1804	20.16	0.603	0.0464	0.00892	19.49	No
7	0.605	0.0463	0.666	0.1159	12.95	0.689	0.0964	0.00722	15.78	No
8	0.690	0.0964	0.723	0.1284	14.34	0.758	0.0945	0.00760	16.60	No
9	0.760	0.0944	0.797	0.1203	13.44	0.829	0.0927	0.00746	16.31	No
10	0.829	0.0927	0.860	0.1075	12.01	0.927	0.0148	0.00617	13.47	No