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Phytochemicals and HPTLC studies of methanolic extract of different germplasms of *Cordia dichotoma* Frost f.

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Abstract

Objective: Most of the traditional medicinal plants in India are not scientifically validated. Scientific evaluation along with traditional knowledge is essential to obtain effective drugs for commercial purposes. To estimate the amount of important secondary metabolites and to establish the fingerprint profile of *Cordia dichotoma* using high performance thin layer chromatography (HPTLC) technique.

Methods: Phytochemical estimation were carried out with standard graph method and HPTLC studies were carried out in one solvent systems, which showed different R_f value.

Results: Secondary metabolites profiling of the extract confirm about the presence of various phytochemicals. HPTLC finger printing of methanol extract of leaf bark and fruits revealed various peaks with R_f values in the range of -0.03 to 0.92.

Conclusion: It can be concluded that different R_f value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals. The study will help in future for identifying this plant for further research.

Keywords: *Cordia dichotoma*, profile, phytochemicals, HPTLC Fingerprinting

1. Introduction

Plants has utility in various way. Each and every part of the plant has applicability, and use for different purposes. On the basis of literature survey number of medicinal uses has been reported by different *Cordia* species (Ioset, 2000, Menezes 2005, Jean-robert 2000) [5, 7, 6]. The ethnopharmacological and chemotaxonomic importance of the genus *Cordia* led us to investigate the chemical and secondary constituent of one of its species.

Cordia dichotoma is a tree of tropical and subtropical regions. It is found in a variety of forests ranging from the dry deciduous forests of Rajasthan to the moist deciduous forests of Western Ghats and tidal forests in Myanmar. In Maharashtra, it grows in moist monsoon forest also. It does not grow gregariously, but is found growing singly in moist shady ravines and valleys. In areas with annual rainfall less than 500 mm, it thrives along streams or depressions where moisture is available. (Patel *et al.*, 2011) [10] The fruits of *Cordia dichotoma* are used as cooling, astringent, emollient, expectorant, anthelmintic, purgative and diuretic agent (Yoganarsimhan 2000) [17]. It is analysed that all the parts of *Cordia dichotoma* used in various treatments, The ripe fruit is used in tonic preparations as well as bark also. The preparation of leaves are useful in infection and headache treatment (Day 1998, Dey 2006).

Herbal products contain a group of phytoconstituents capable of variation. The plant constituents has variability within the same plant. The variability may be from grower to grower, crop to crop and also depends on the harvest and post-harvest handling. Apart from modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. (WHO, 1998). The phytochemicals are of considerable physiological and morphological importance in plants. Plants contain phenolic components, has important properties like anti-allergenic, anti antherogenic, anti-inflammatory and antimicrobial, antioxidant, anti-thrombotic, cardio protective and vasodilatory effects (Benavente-Garcia 1997, Manach and Mazur 2005, Samman 1998) [3, 11].

Sophisticated modern technique of standarization such as TLC and HPTLC provide quantitative and semi-quantitative information about the main active constituents or marker compounds present in the crude drug or herbal drugs (Banu Nagranjan 2014) [2]. The chromatographic techniques and marker compounds which are used to standaridize.

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Ayurvedic formulations has limitations because of variable sources, chemical complexity and the current research documented that many phytochemicals are protect humans against diseases. The phytochemicals present in leaves, bark, fruits, work differently. The need of scientific validations of these phytochemicals is required.

Chromatographic fingerprinting technique are most significant method which can be used for the routine herbal drug analysis and for quality assurance. The scientific validation performed by various technique among that HPTLC s predominate. The WHO has emphasized to ensure the quality of medicinal plant products using modern controlled technique like HPTLC. HPTLC offers better resolution and estimations of active constituents can be done with reasonable accuracy in a short time (Sasikumar *et al.*, 2009, Ojha and Kumar 2012, Mona *et al.*, 2012, Kulkarni *et al.*, 2013) [12, 9].

Materials and Methods

Plant Material: The plant material was collected from different geographical region of Nanded district. The ten genotypes was selected on the basis of morphological variations. Few of the genotypes was selected from forest area and few of them was selected from farmers field. Ten genotypes were named as NCd1 to NCd10. N were abbreviated as Nanded and Cd for *Cordia dichotoma*. (Table.1.)

Preparation of extract

Fifty gm of powdered leaves, bark and fruit tubers of *C. dichotoma* were extracted with 250 ml methanol at the temperature between 60 and 65 °C for 24 h by using a soxhlet extractor. The solvent was evaporated by rotary evaporator to obtain viscous semi-solid masses. This semi-dry methanolic crude extract was subjected to phytochemical estimation and HPTLC analysis.

Phytochemical Screening

Qualitative Analysis of Phytochemicals

In present study five secondary metabolites was analysed qualitatively and Quantitatively. The extracts of the dry powdered of leaves, fruit and bark of *Cordia dichotoma* was analyzed for the presence of various phytoconstituents like alkaloid, phenol, flavonoid, saponin and tannin.

Test for alkaloids

A) Wagner's Test: 3-4 drops of Wagners reagent was added to 2 mg of methanolic extract acidified with 1.5%v/v hydrochloric acid. Alkaloid presence was confirmed after the formation of yellow or brown precipitate. (Sofowara 1993, Trease and Evans 1989, Harborne 1973) [14, 15, 4].
(Wagners reagent for alkaloids- To 100ml of distilled water 6 g of potassium iodide and 2g of iodine was dissolved

B) Mayer's Test: To 2mg of methanolic extracts 3-4 drops of the Mayer's reagent was added. Alkaloids presence was confirmed after the formation of white or pale yellow precipitate.

(Mayer's reagent –To 60 ml of distilled water 1.35g mercuric chloride was dissolved and pours into 10ml of distilled water

contained 5g potassium iodide. Total volume was made 100ml)

Test for Flavonoids

A) Shinoda's Test: To 5 ml of ethanol 2 mg of methanolic extract was dissolved to this 10 drops of dilute hydrochloric acid was added, it was followed by adition of small piece of magnesium. Flavonoids presence was confirmed after the formation of brown reddish or pink color. (Sofowara 1993, Trease and Evans 1989, Harborne 1973) [14, 15, 4].

B) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test for Phenols

A) Ferric chloride Test: The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution is added. A dark green colour indicates the presence of phenolic compounds. (Sofowara 1993, Trease and Evans 1989, Harborne 1973) [14, 15, 4].

Test for Tannin

A) Ferric chloride Test: About 1 ml of the mehanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration (Sabri *et al.*, 2012)

B) Lead acetate Test: 1ml of the different filtrate was added with three drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins. (Singh *et al.*, 2012) [13]

Test for Saponins

A) Foam Test: A drop of sodium bicarbonate was added in 5ml of methanolic extract in the test tube. Later on test tube was shaken vigorously and kept test tube stand for 3 minute. Saponin presence was confirmed after the formation of honeycomb like froth.

B) Hemolytic Test: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins. (Sofowara 1993, Trease and Evans 1989, Harborne 1973) [14, 15, 4]

HPTLC (High Performance Thin Layer Chromatography) profile

Sample Preparation

Methanolic extracts obtained were evaporated under reduced pressure using rotovac evaporator. Each extract residue was re-dissolved in 1ml of chromatographic grade methanol, which was used for sample application on pre-coated silica gel 60F254 aluminum sheets. (Wagnoor 1995) [16]

Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent Ethylacetate: (10): formic acid (1.1): Gl. acetic acid (1.1): water (2.6) for methanolic extracts.

Sample Application

Application of bands of each extract was carried out (4 mm in length and 1 μ l in concentration for Extract) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F254 Aluminum sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with solvent Ethyl acetate (10): formic acid (1.10): Gl. acetic acid (1.1): water (2.6) for 15 minutes.

Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light (Plate XVI). The chromatograms were scanned by densitometer at 254 and 366nm for methanolic extract. The R_f values and finger print data were recorded by WINCATS software. (Wagnor 1995, Harborne 1973, Banu and Nagranjan 2014) [16, 4, 2]

Results

Phytochemical screening of extracts of leaves, bark and fruits for alkaloid, phenol, flavonoid, tannin and saponin was measured with different qualitative tests. Alkaloid screening

was carried by using Wagners Test and Mayers Test, flavonoid screening by using Shinoda's Test and Alkaline Reagent Test, Phenol screening by using Ferric chloride Test, Tannin by using Lead acetate Test and Ferric chloride Test and Saponin screening by using Foam Test and Haemolytic Test Extract of leaves and fruits of NCd2, NCd4, NCd7, NCd8, NCd9 shows strong coloration (Table. 2.) The comparative screening of these phytochemicals was observed. Comparing between leaves fruit bark and fruit, leaves and fruit indicate strong colouration. Based on present observation among the test for alkaloid- Mayers test, for flavonoid -ferric chloride test, for phenol- lead acetate test, for tannin- lead acetate test was preferred. Among the tested phytochemicals flavonoid and phenol gives good result.

In the study total eight methanolic extracts of *Cordia dichotoma* were used. The extracts were of plant parts consisting of leaves, bark and fruits. The quercetin was used standard flavonoid. These extracts of *Cordia dichotoma* were subjected to HPTLC analysis by specific solvent system ethyl acetate: Formic acid: Acetic acid: Water (10:1.1:1.1:2.6) and detected under UV at 254 and 366 nm. Blue, brown color zone was detected in UV after derivatization in the chromatogram indicates the presence of polyphenols. The R_f values ranged from -0.03 to 0.61. The standard quercetin shows maximum R_f value was 0.92 and were found to be more predominant as the area is more with 92.36% Hence it is quercetin.

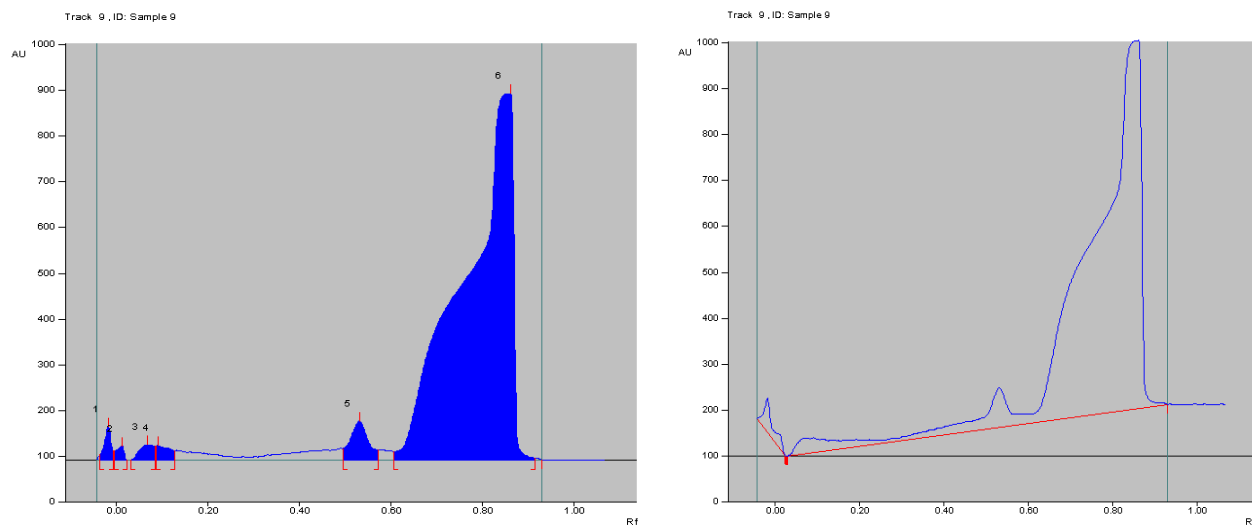


Fig: (Track 9 sample 9) HPTLC of standard Quercetin

The present study includes 10 germplasms of *Cordia dichotoma* collected from different geographical location of Nanded district. There was total 30 extracts obtained from leaves, bark and fruits of 10 germplasms. Among the 30 extracts only 3 extracts of leaves, 1 extract of bark and 4 extract of fruits was sorted for the HPTLC analysis. Total 9

samples was subjected for HPTLC. In that track no. 1 was standard quercetin and from track 2 to 9 was extracts. (Fig.4) The leaves extracts of *Cordia dichotoma* gives various peaks ranged from 6 to 9. The peaks indicates the various phytoconstituents in extracts. (Fig.1)

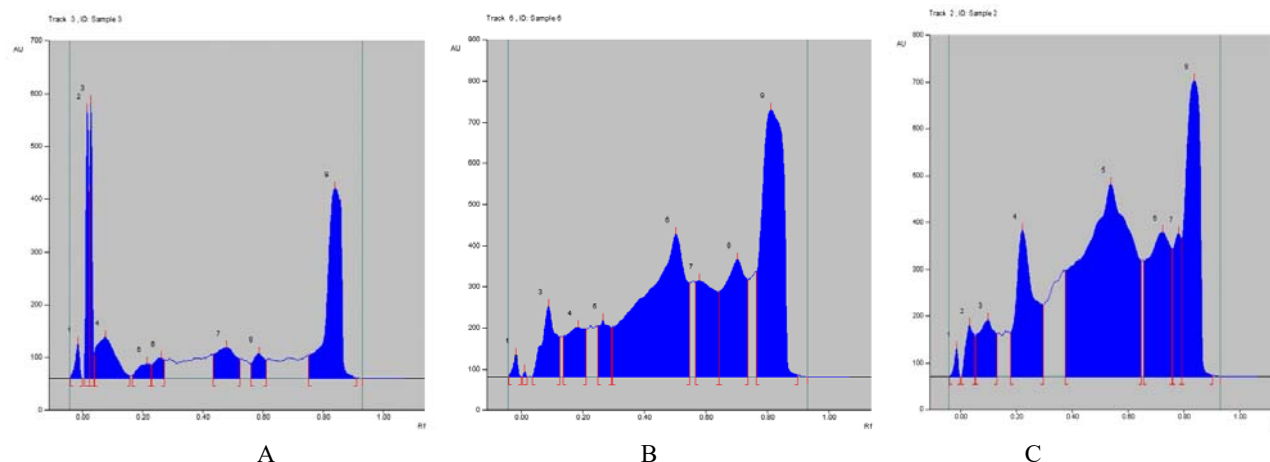


Fig 1: HPTLC of methanolic extract of leaves extract (A) NCd4, (B) NCd7, (C) NCd8 of selected germplasm of *Cordia dichotoma*.

The bark extracts of *Cordia dichotoma* gives various 11 peaks. The peaks indicates the various phytoconstituents in extracts. (Fig 2)

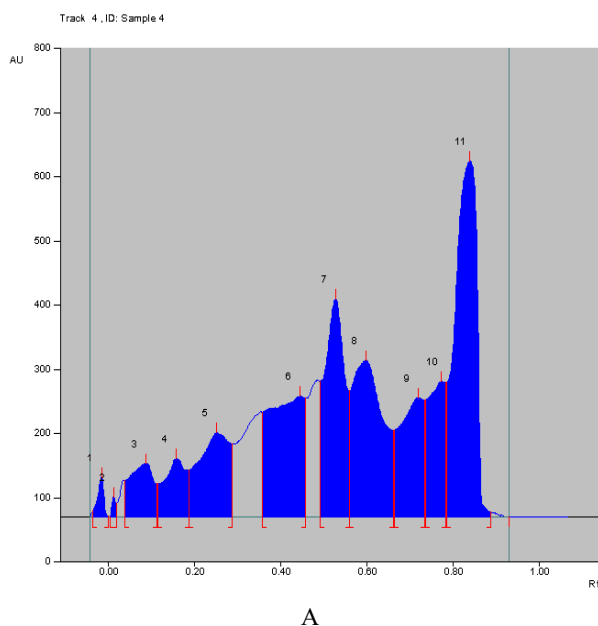


Fig 2: HPTLC of methanolic extract of bark extract (A) NCd4 of selected germplasm of *Cordia dichotoma*.

The fruits extracts of *Cordia dichotoma* gives peaks ranged from 6 to 12 the peaks indicates the various phytoconstituents in extracts. (Fig 3)

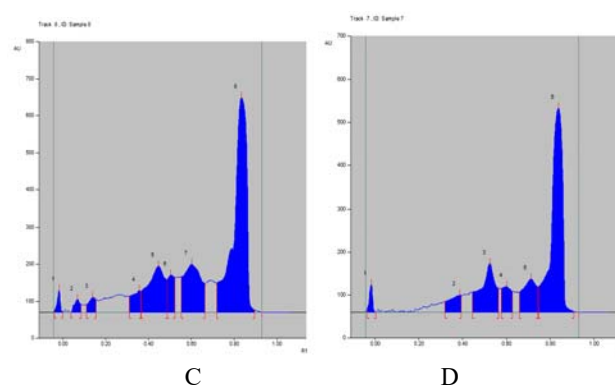
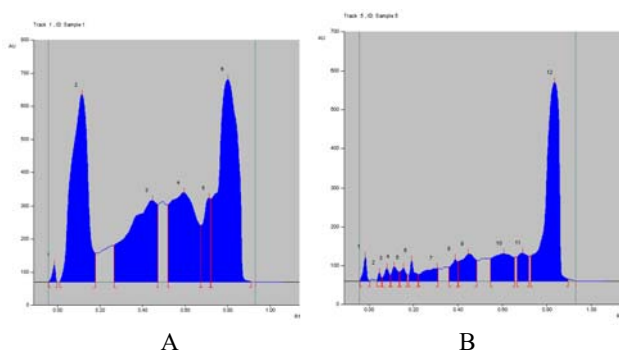


Fig 3: HPTLC of methanolic extract of fruits extract (A) NCd2, (B) NCd4, (C) NCd7, (D) NCd9 of selected germplasm of *Cordia dichotoma*.

Discussion

The extract shows moderate result compared with other plants secondary metabolite. The ferric chloride and lead acetate test of flavonoid for selected extract gives more strong reaction, while comparing in germplasms all the three extracts of NCd2 (Mudhkhed), NCd7 (Pawdiwadi) show strong +ve reaction, while comparing in between the leaves, bark and fruit extract leaves and fruit extract gives +ve reaction for all the five secondary metabolites. The screening of phytochemicals is important to carry out further quantitative estimation of these metabolites. Comparing in the selected metabolites the saponin gives very weak reaction in all extracts of leaves, bark and fruit of *C. dichotoma* germplasms, and also tannin shows weak colouration in all the extract of germplasms. (Table. 2.)

Nawal (2011) [8] and Allahadi (2015) [1] documented that the phyto-chemical screening showed the presence of saponin, coumarins, triterpens and flavonoids in different parts of *Cordia Africana*. The result of present study showed that, in few parts of plant the sterols gives positive result while negative in other parts. The concentration of phytochemicals were ranged from high in stem, moderate in bark and low in leaves and fruits, they isolated, some of the secondary metabolites like flavonoids, sterols, saponins from *Cordia sinesis*.

The specificity of the method was ascertained, by analyzing standard and sample spectral revealed that the peaks obtained

from both the standard and test samples were identical. (Banu and Nagarajan 2014) [2]. The pharmacognostical parameter including HPTLC are helpful for the future identification and authenticity of this tree in the herbal industry. The physical characteristics, such as loss on drying, ash values and extractive values will be helpful to identify the authenticity of the drug, by using powdered material. It will serve as a standard data for the quality control of the preparations containing *Cordia dichotoma*

Herbal medicines are composed of many phyto constituents and therefore it is capable of variation. Due to variation, the

HPTLC obtains the reliable chromatographic fingerprint profile represent pharmacologically active components. HPTLC is very important parameter of herbal drug and medicine, and also for proper identification of medicinal constituents.

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Table 1: Details of different genotypes of *Cordia dichotoma* populations and collection sites in Nanded district of Maharashtra

Sr. No.	Accession No.	Village/district	Collection site	Latitude 'N'	Longitude 'S'
1	NCd1	Ardhapur/Nanded	Farmers' field	19.28	77.37
2	NCd2	Mudkhed/Nanded	Farmers' field	19.14	77.49
3	NCd3	Barad/Nanded	Farmers' field	19.18	77.42
4	NCd4	Tamsa/Nanded	Forest area	17.40	78.48
5	NCd5	Vishnupuri/Nanded	University campus	19.10	77.28
6	NCd6	Bhokar/Nanded	Farmers' field	19.22	77.67
7	NCd7	Pawadiwadi/Nanded	Farmers' field	19.15	77.33
8	NCd8	Limbgaon/Nanded	Farmers' field	19.17	77.20
9	NCd9	Patnoor/Nanded	Farmers' field	17.22	78.32
10	NCd10	Vishnupuri /Nanded	University campus	19.10	77.28

Table 2: Phytochemical Screening of methanolic extract of leaves, bark and fruits of selected germplasm of *Cordia dichotoma* from Nanded district in Maharashtra.

Sr. No.	Accession No.	Methanol extracts	Alkaloid		Flavonoids		Phenol	Tannins		Saponin	
			A	B	C	D	E	F	G	H	I
1	NCd1	Leaves	-	+	+	+	+	+	+	-	+
		Bark	-	-	-	+	+	+	-	-	-
		Fruit	-	+	++	+	+	-	+	-	+
2	NCd2	Leaves	+	++	+	+	+	+	+	-	-
		Bark	-	+	+	+	+	-	+	-	+
		Fruit	-	+	++	++	++	-	+	-	+
3	NCd3	Leaves	+	+	-	+	-	+	-	+	+
		Bark	-	-	+	-	+	-	-	-	-
		Fruit	++	+	++	+	+	-	+	-	+
4	NCd4	Leaves	+	++	++	+	++	++	+	+	+
		Bark	+	+	+	-	+	+	+	-	+
		Fruit	+	++	++	++	++	+	++	-	++
5	NCd5	Leaves	-	+	+	-	+	+	-	+	-
		Bark	-	-	-	-	+	-	+	-	-
		Fruit	+	+	+	-	+	-	-	-	+
6	NCd6	Leaves	+	+	+	+	-	++	-	+	+
		Bark	+	-	+	-	+	-	-	-	-
		Fruit	+	+	+	+	+	-	+	++	+
7	NCd7	Leaves	-	++	++	++	++	-	++	+	+
		Bark	-	+	+	+	+	-	+	-	+
		Fruit	+	++	++	++	++	+	++	-	+
8	NCd8	Leaves	-	++	++	+	++	-	+	+	+
		Bark	-	-	+	-	+	-	-	-	-
		Fruit	-	+	+	+	+	+	+	+	+
9	NCd9	Leaves	-	+	++	+	++	-	+	+	+
		Bark	-	+	+	+	+	-	+	-	+
		Fruit	+	+	++	++	++	+	+	-	+
10	NCd10	Leaves	-	+	+	+	+	+	+	+	+
		Bark	-	-	-	-	+	-	-	-	-
		Fruit	++	-	+	+	+	-	+	-	+

++Strong coloration,

+Weak coloration,

- Absent

A-Wagner's Test, B- Mayer's Test, C- Shinoda's Test, D- Alkaline Reagent Test E- Ferric chloride Test, F- Ferric chloride Test, G- Lead acetate Test, H-Foam Test, I- Haemolytic Test.

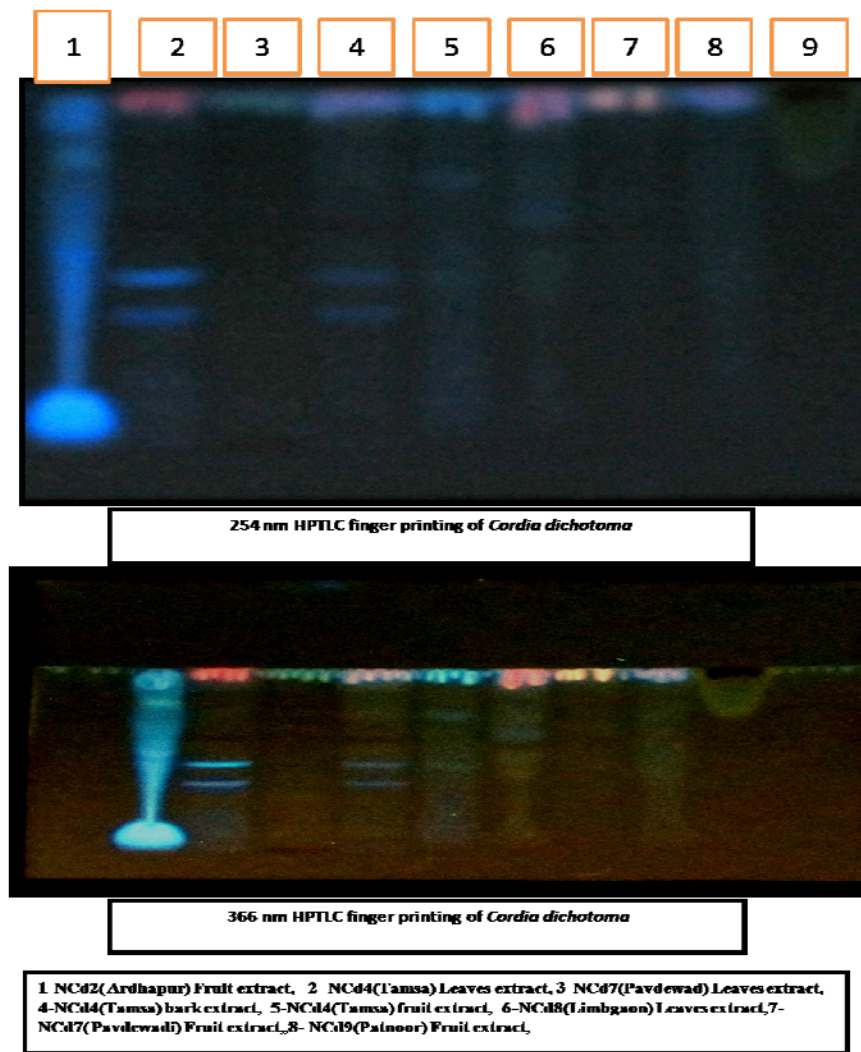


Fig 4: HPTLC finger printing of extracts of *C. dichotoma*.

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