Research Article

TLC and HPTLC Analysis of *Dodonaea viscosa* Leaf and *Wrightia tinctoria* Fruit Extracts

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ABSTRACT

The Present study is planned to Investigate the chemical compounds present in selected Plant drugs *Dodonaea viscosa* and *Wrightia tinctoria* chosen from ethnic information. Presence of phenolics, flavonoids and steroids was assessed by TLC using various mobile phases like and Chloroform: Methanol-9:1, Ethyl acetate: Methanol: Water-80:40:8, Acetic acid and Chloroform-1:9,Ethyl acetate: Benzene-9:11,Acetic acid: Conc HCl: Water-30:3:10. HPTLC fingerprinting of phenolic compounds of the extracts of *WT* fruit and *DV* leaf at 254 nm in solvent system-(1) acetic Acid: chloroform (1:9), solvent system (2)Ethyl acetate: Benzene-9:11,solvent system (3) Ethyl acetate: Methanol: Water-80:40:8 has been presented.

Keywords: TLC, HPTLC, *Wrightia tinctoria*, *Dodonaea viscosa*, analysis.

INTRODUCTION

Ethical analysis of global herbal medicine research pose multiple scientific questions that shed light on the difficulties of conducting research with herbal medicines worldwide. Finding appropriate ways to conduct this type of research is an ongoing challenge[1].

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous animals. Atleast 12,000 such compounds isolated so far; a number estimated to be less than 10% of the total Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus, herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology.[2,3]

Modern medicine which was derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant. 4 some of the pharmaceuticals currently available to physicians are derived from plants that have a long history of use as herbal remedies. Examples of such drugs include aspirin, digoxin, quinine and opium.[4]

Compounds derived from Extracts which can be used as drugs in future do need a detailed study in the light of modern medicine in bringing new herbal chemical entities. Hence there is necessity of Finger printing analysis/ chromatography**.**

Plant Material

***1 Wrightia tinctoria* (WT)** is a small deciduous tree which grows up to a height with pale grey, smooth, thin bark abounding in yellow milky juice with opposite branches; leaves are simple, opposite, elliptic and ovate, acuminate, main nerves 6-12 pairs; flowers are white, fragrant, terminal cymes; fruit follicles in pairs, pendulous, cylindrical, tips adhering at first, seeds linear, pointed at the apex with deciduous

1.1 Ethno medicinal uses: Flowers, fruits and seeds eaten as vegetables. The juice of unripe fruits is used for antihelminthic leaves chewed with salt to relieve toothache. Leaves and Bark used to treat psoriasis, stomach pain, dysentery, toothache. [5,6,7]

1.2 Phyto chemical review on *Wrightia tinctoria:* Khyadae et al., made a comparative phytochemical investigation of the bark of *Wrightia tinctoria* and *Wrightia arborea* and indicated the presence of alkaloids, phenolics, saponins and tannins in both the species, but shown the presence of terpenoids only in *Wrightia tinctoria* while flavonoids in arboria species. Mahendra et al., in the study showed the presence of various compounds such as alkaloids, flavanoids, phlobatannin, phenolics, steroids, saponins, tannins and terpenoids in leaf extract of *Wrightia tinctoria* Sathianarayanan S et al., has performed preliminary phytochemical investigation of aqueous and alcoholic extract of *Wrightia tinctoria* whole plant and showed presence of carbohydrates, phytosterols tannins and lignins. Akihisha et al., isolated identified a new sterol 14 α-methyl zymosterol from unsaponified lipid of *Wrightia tinctoria* seed and four uncommon sterols in addition to other phytosterols. Jain et al., identified and characterized the bioactive principles lupeol, stigmasterol and campestrol from the petroleum ether extract of woody stem of *Wrightia tinctoria* subjected to chromatographic techniques and spectroscopic method.[8,9,10]

***2 Dodonaea viscosa (DV)* Linn.-** Family Sapindaceae. The genus *Dodonaea* is restricted to Australia, but it is distributed throughout the tropics, subtropics and

temperate regions of India, Africa, and Mexico. It is a multi stemmed or single-stemmed small shrub or tree; rough blackish bark, twigs glandular reddish-brown in color, Leaves -alternate, simple, estipulate with a very short petiole, margins entire with apiculate apex, with 15–20 pairs of lateral veins. Flowers bisexual or unisexual, greenish-yellow to red in color; Fruit is papery, light red - colored to brown, splits along central septa (dehiscent), Seeds, black, 2 seeded, compressed, subglobulose, 3 mm in diameter.

2.1 Ethno medicinal uses*: Dodonaea viscosa* has many healing properties, hence traditionally used to various ailments by native people from all regions where it is found. Infusions made from Stems used in fevers, rheumatism leaves used as a painkiller to soothe toothaches and headaches Decoction of the leaves orally-administered for Digestive system disorders. Decoction of roots, are taken by East Africa women to stimulate milk production and for the treatment of dysmenorrheal.[11,12,13]

2.2 Phyto chemical review on *Dodonaea viscosa:* Kusum sachdev et al., isolated a new flavonoid Aliarin from *DV* Linn and established the structure as 5, 7, 4,-trihydroxy-3,-(3- hydroxyl methyl butyl) -3, 6-dimethoxy flavones by chemical and spectroscopic analysis. Venkateshwara Rao performed a preliminary study and resulted in isolation of stigmasterol and β-sitosterol from the unsaponified fraction of benzene extract of leaves and also isolated isorhamnetin from ethanolic extract. Ramachandran Nair et al., isolated isorhamnetin-3-o-rutinoside, quercetin-3-o-galactoside and quercetin-3-o-rutinoside from the leaves and pods of the *Dodonaea viscosa* ethanol extract. Rachel Mata et al., reported and elucidated a new diterpene and a novel coumaric acid ester along with other known compounds from aerial parts of *Dodonaea viscosa* including structures determined by spectroscopic analysis and chemical reactions. Kusum sachdev et al., isolated a new flavonoid Viscosol from the aerial parts of *Dodonaea viscosa* and the structure was established on the basis of spectral studies by the conversion of flavonol penduletin into per methyl viscosol. Kusum sachdev et al., Performed studies on chloroform portion of ethanolic extract which lead to isolation of a diterpenoid a new compound designated as dodonic acid. The structure and stereochemistry of dodonic acid have been established by chemical and spectroscopic means. Ghisalberti et al., identified and characterized flavones from seeds, bark, flowers and leaves of *Dodonaea viscosa* ·Siddiqui's isolated flavonoidal glycosides of quercetin and isorhamnetin. Getie et al., isolated quercetin, kaempferol and isorhamnetin from the *Dodonaea viscosa* leaf extract. Wagner et al., Chromatographically pure Dodonosides A and B (saponin ester mixture) were isolated and reported.[14-17]

TLC and HPTLC analysis: [18,19,20]

Standardization of herbal drugs/extracts is an important and challenging step in the determination of specific pharmacological activities, because the pharmacological activity is not alone dependent on one active constituent of the plant, but due to synergistic effect of all the constituents of the plant. Chromatographic trials were carried out for the phenolics and steroids.

Thin Layer Chromatographic Studies:

TLC studies of crude Methanolic and the ethyl acetate and chloroform extracts of the *WT* and *DV* respectively were carried out in various mobile phases and tried for good resolution. The precoated silica gel G 60 F254TLC plates were used (Merck).

Sample application: With the help of capillary sample was applied on to the TLC plates about 2 cm above the bottom of the plate.

Chamber saturation: Mobile phase is transferred into the chamber and allowed for saturation for about 30 minutes.

Development of chromatogram: After chamber saturation TLC plate containing the sample spot was placed in the chamber and solvent was allowed to run to on the plate and the solvent front was noted.

Detection: The detection was done by UV examination and spraying specific reagents. Qualitative analysis was done by calculating the R_f value.

The following solvent systems and spray reagents were used for the detection of phytoconstituents

Solvent systems:

Chloroform: Methanol-9:1

Ethyl acetate: Methanol: Water-80:20:8

Acetic acid and Chloroform-1:9

Ethyl acetate: Benzene-9:11

Acetic acid: Conc HCl: Water-30:3:10

Spray reagents:

Vanillin –sulphuric acid reagent

Solution1: 1% ethanolic vanillin

Solution 2:10% ethanolic sulphuric acid

The plate was sprayed vigorously with solution1, immediately followed by solution 2. Then heated for 5 to 10 min at 110ºC.

5-10% Ferric chloride reagent- A 5% w/v solution of ferric chloride in 90% alcohol.

High Performance Thin layer Chromatography:

HPTLC studies were performed using Camag HPTLC system equipped with Linomat V applicator, Camag TLC scanner and WInCATS-4 software for interpretation of data. The aluminium plate (10x10 cm) precoated with silica gel $60F_{254}$ (Merck) 2 µl of the sample were loaded as 7 mm band length in 10x10 silica gel G 60F 254 TLC plate using Camag Linomat V applicator using a Hamilton syringe. The loaded plate was kept in TLC developing chamber with

respective mobile phase and developed up to 8.5 cm. The plate was kept in scanner and scanned at 254 nm. The peak table and peak desitogram was identified. The extracts of the *WT* fruit and the *DV* leaf were subjected to TLC and HPTLC. HPTLC fingerprints were obtained for the phenolics and the steroidal compounds. In fingerprint chromatography, plant extracts are dissolved in solvents like methanol or water-alcoholic solvent. The extract is run through a mobile phase and then separated by measuring affinities towards the adsorbent.

RESULTS

Thin Layer Chromatography of *D.viscosa and w.tinctoria (DV and WT)*

In the present study the TLC and HPTLC finger print profiles of the *WT* fruit and the *DV* leaf extracts were determined for the bio-marker compounds. HPTLC fingerprints were obtained for the phenolics and the steroidal compounds.

DVC-*Dodonaea viscosa* Chloroform Extract

DVM-*Dodonaea viscosa* Methanolic extract

WTM-*Wrightia tinctoria* methanolic extract

WTEA-*Wrightia tinctoria* Ethyl acetate extract

Table 1: TLC studies of *D.viscosa* and *W.tinctoria*

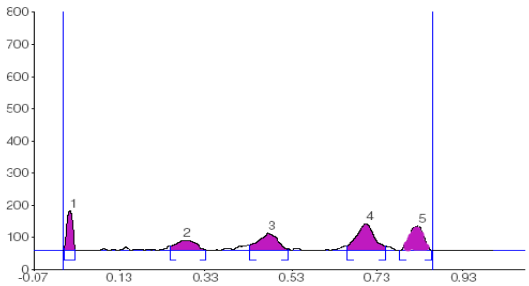
Solvent system	Number of spots				Rf values				Phytoconstituents	Detection
	DVM	DVC	WTM	WTEA	DVM	DVC	WTM	WTEA		
1.Acetic acid : Chloroform (1:9)	3	2	3	2	0.48(bluishpink), 0.70(blue), 0.83l(blue)	0.49(red), 0.70(pink)	0.54(pink), 0.65(red), 0.77(red)	0.50(pink)' 0.60(red)	phenolic acids/steroids	Vanillin-H_2SO_4/$FeCl_3$/ ammonia vapour
2.Ethyl acetate: Benzene (9:11)	4	3	3	3	0.62(pink), 0.82(blue), 0.90(blue), 0.70(blue)	0.82(blue), 0.91(blue), 0.46blue)	0.56(pink), 0.78blue), 0.92(blue)	0.91(blue) 0.77(grey) 0.46(pink)	phenolic acids and simple phenols	Vanillin-H_2SO_4/$FeCl_3$ /ammonia vapour
3. Ethyl acetate: methanol: water (80:40:8)	3	1	2	1	0.79(blue), 0.65(Pink) 0.42(purple)	0.62 (purple)	0.78 (purple) 0.79(blue)	0.4(violet)	Phenolics and steroidal/ triterpenoids	Vanillin-H_2SO_4/$FeCl_3$

HPTLC analysis:

Figure 1: HPTLC fingerprinting of phenolic compounds of the extracts of *WT* fruit and *DV* leaf at 254 nm In solvent system, acetic Acid: chloroform (1:9)

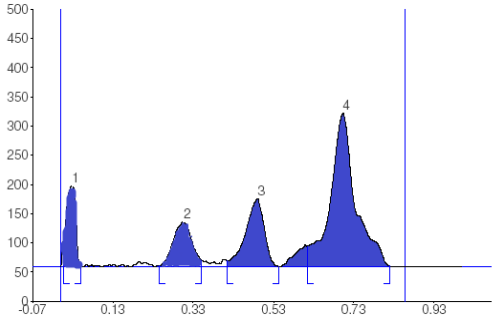
Track 1, ID: DVM

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	4.9	0.01	124.4	33.32	0.03	4.5	1340.9	14.18
2	0.25	13.6	0.28	32.3	8.66	0.33	1.9	1244.8	13.16
3	0.43	17.7	0.48	54.6	14.62	0.52	4.8	1968.5	20.82
4	0.66	14.9	0.70	84.5	22.65	0.75	13.7	2852.7	30.17
5	0.78	1.0	0.83	77.4	20.75	0.86	0.4	2049.0	21.67



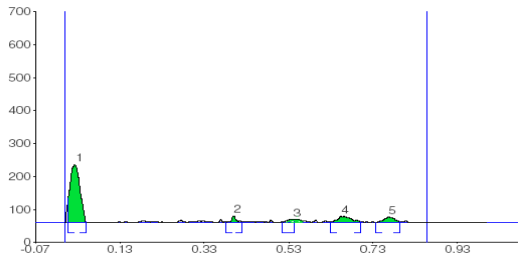
Track 2, ID: DVC

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	54.3	0.03	137.9	23.24	0.05	0.3	2626.2	11.35
2	0.25	0.6	0.31	76.3	12.86	0.35	10.4	2864.4	12.38
3	0.42	8.9	0.49	116.6	19.65	0.54	0.0	4321.6	18.68
4	0.61	34.1	0.70	262.5	44.24	0.82	0.5	13324.6	57.59



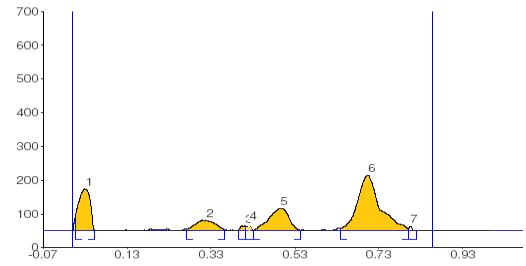
Track 3, ID: WTM

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	74.6	0.02	176.9	71.36	0.05	2.9	3342.7	72.26
2	0.38	2.3	0.40	22.0	8.86	0.42	2.1	202.8	4.38
3	0.52	2.2	0.54	10.2	4.12	0.54	10.2	144.6	3.13
4	0.63	2.3	0.65	20.6	8.31	0.70	4.1	551.5	11.92
5	0.74	0.1	0.77	18.2	7.35	0.79	4.1	384.1	8.30



Track 4, ID: WTEA

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	49.4	0.03	125.3	28.08	0.05	0.6	2794.6	19.46
2	0.27	5.9	0.32	32.0	7.17	0.36	4.0	1266.3	8.82
3	0.39	0.6	0.41	15.5	3.48	0.41	15.5	140.6	0.98
4	0.41	15.0	0.42	25.6	5.74	0.43	2.0	184.4	1.28
5	0.43	2.0	0.50	67.5	15.12	0.54	2.3	2626.2	18.28
6	0.64	7.9	0.70	165.5	37.09	0.80	5.8	7275.2	50.65
7	0.80	6.5	0.81	14.8	3.32	0.82	0.0	77.3	0.54



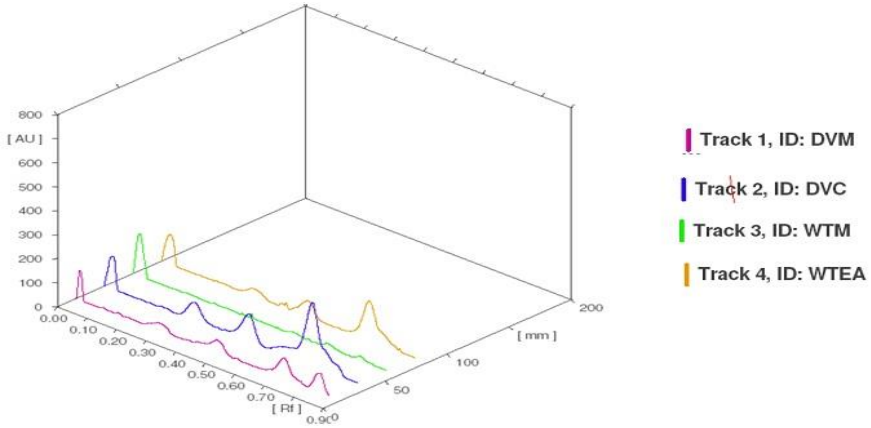
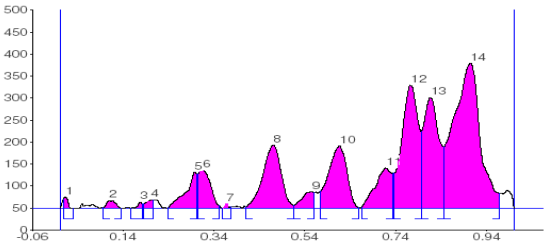


Figure 2: HPTLC fingerprinting of phenolic compounds of the extracts of *WT* fruit (Methanolic and Ethyl acetate)and *DV* leaf (Methanolic and Chloroform)at 254 nm in solvent system B ethyl acetate: benzene (9:11)

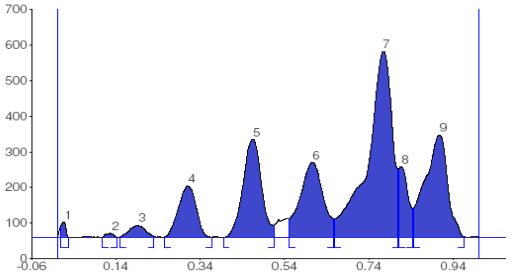
Track 1, ID: DVM

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	19.2	0.01	25.4	1.66	0.03	0.0	221.6	0.40
2	0.09	1.6	0.11	17.9	1.17	0.13	0.1	320.1	0.58
3	0.16	0.0	0.17	15.2	0.99	0.18	9.2	122.0	0.22
4	0.18	9.3	0.20	19.7	1.29	0.20	18.9	293.0	0.53
5	0.24	0.5	0.30	81.7	5.33	0.30	76.8	1842.6	3.35
6	0.30	77.4	0.31	87.5	5.70	0.35	6.7	2194.1	3.99
7	0.36	0.1	0.37	10.9	0.71	0.38	3.8	71.2	0.13
8	0.41	2.5	0.47	143.6	9.36	0.51	6.3	5375.9	9.79
9	0.52	6.6	0.56	37.6	2.45	0.56	35.0	953.6	1.74
10	0.57	39.1	0.62	141.8	9.24	0.66	1.6	5254.8	9.57
11	0.66	0.9	0.72	91.7	5.98	0.73	78.8	3051.3	5.56
12	0.73	79.8	0.77	279.4	18.21	0.80	174.1	9467.1	17.24
13	0.80	174.6	0.82	251.8	16.41	0.84	138.9	8004.0	14.57
14	0.85	139.6	0.90	329.7	21.50	0.97	33.6	17754.4	32.32



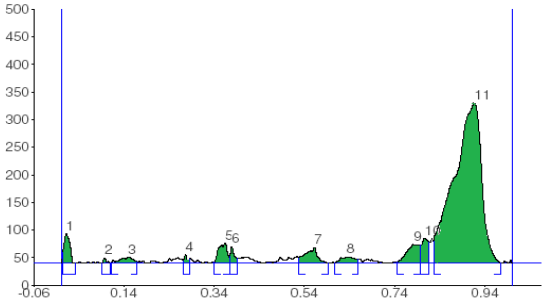
Track 2, ID: DVC

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	27.4	0.01	43.8	2.53	0.03	0.1	459.1	0.60
2	0.11	1.0	0.13	10.8	0.62	0.14	0.2	195.3	0.26
3	0.15	1.3	0.19	34.8	2.01	0.23	3.5	1203.5	1.58
4	0.25	1.4	0.31	145.2	8.38	0.36	0.1	5684.9	7.46
5	0.39	0.0	0.46	275.6	15.91	0.51	33.9	11259.1	14.77
6	0.55	52.4	0.60	211.4	12.20	0.66	52.4	10668.0	14.00
7	0.66	52.5	0.77	523.2	30.20	0.81	190.6	28310.9	37.15
8	0.81	191.8	0.82	199.1	11.49	0.84	80.6	4188.2	5.50
9	0.84	81.6	0.91	288.4	16.65	0.96	2.6	14235.9	18.68



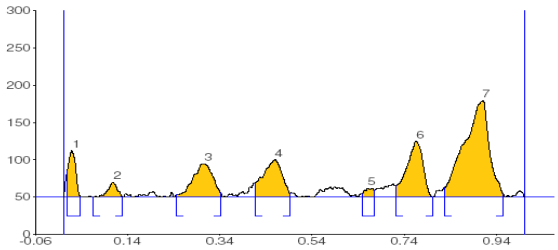
Track 3, ID: WTM

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	19.4	0.01	53.7	9.37	0.03	0.2	648.2	3.07
2	0.09	0.8	0.10	10.5	1.83	0.11	0.2	64.3	0.30
3	0.11	0.7	0.15	11.4	1.99	0.17	2.8	307.7	1.46
4	0.27	5.2	0.28	16.4	2.87	0.28	7.2	105.0	0.50
5	0.34	1.8	0.36	37.4	6.52	0.37	11.7	646.6	3.07
6	0.37	13.4	0.38	30.7	5.36	0.39	9.1	240.4	1.14
7	0.53	9.9	0.56	29.0	5.06	0.59	0.4	719.0	3.41
8	0.61	0.6	0.63	12.6	2.21	0.66	6.6	355.1	1.68
9	0.75	2.2	0.78	34.8	6.06	0.80	33.3	902.3	4.28
10	0.80	33.4	0.81	45.5	7.93	0.82	38.9	683.6	3.24
11	0.83	47.9	0.92	291.2	50.80	0.97	0.4	16415.0	77.84



Track 4, ID: WTEA

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	35.6	0.02	62.6	15.80	0.04	0.6	906.5	6.35
2	0.06	0.0	0.11	20.4	5.16	0.13	0.3	377.7	2.65
3	0.25	3.1	0.30	45.0	11.35	0.34	6.1	1803.1	12.63
4	0.41	18.5	0.46	50.6	12.77	0.49	5.5	1951.1	13.67
5	0.65	4.0	0.66	12.4	3.14	0.67	9.6	210.2	1.47
6	0.72	16.0	0.77	75.3	18.99	0.80	0.3	2466.5	17.28
7	0.83	12.9	0.91	130.0	32.80	0.95	4.8	6557.7	45.95



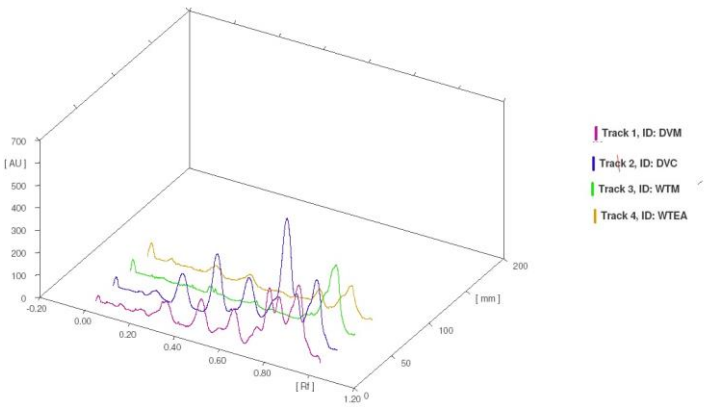
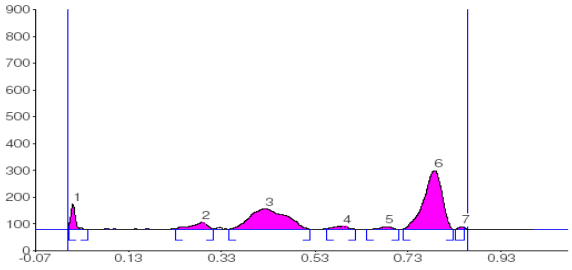


Figure 3: HPTLC fingerprinting of phenolic compounds of the extracts of *WT* fruit and *DV* leaf at 254 nm In solvent system ethyl acetate: methanol: water (80:40:8)

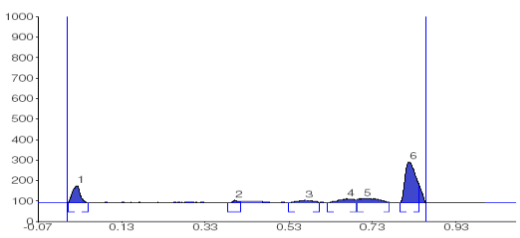
Track 1, ID: DVM

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	12.8	0.01	95.5	20.91	0.04	0.2	765.9	5.36
2	0.23	3.6	0.29	27.9	6.10	0.31	2.7	783.8	5.48
3	0.35	0.4	0.42	77.6	16.99	0.52	1.4	4991.6	34.92
4	0.56	3.0	0.59	11.7	2.56	0.62	0.4	329.9	2.31
5	0.64	0.0	0.68	11.5	2.52	0.71	0.6	242.0	1.69
6	0.72	2.3	0.79	221.5	48.49	0.83	0.4	7078.4	49.52
7	0.83	0.2	0.85	11.1	2.44	0.85	9.2	101.6	0.71



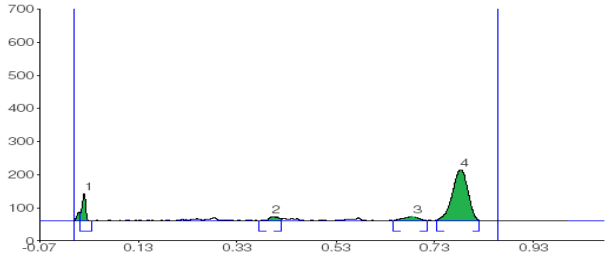
Track 2, ID: DVC

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	1.3	0.02	85.4	23.61	0.05	0.9	1278.5	16.70
2	0.38	0.9	0.40	14.3	3.96	0.41	7.1	145.4	1.90
3	0.53	0.6	0.57	14.1	3.90	0.60	1.6	441.5	5.77
4	0.62	1.5	0.67	22.3	6.16	0.69	15.8	638.4	8.34
5	0.69	16.4	0.71	23.2	6.42	0.77	1.6	931.6	12.17
6	0.80	1.2	0.82	202.3	55.95	0.84	84.3	4219.1	55.12



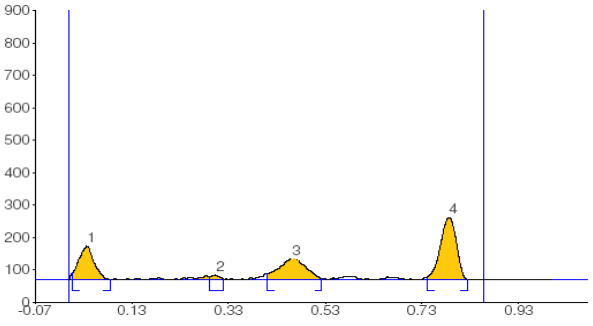
Track 3, ID: WTM

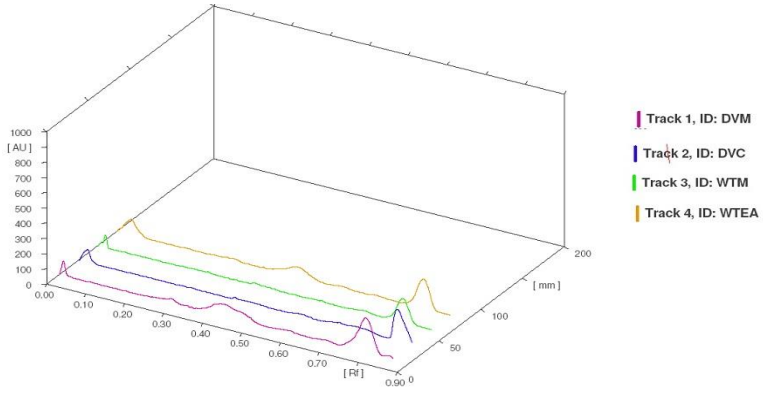
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	24.8	0.02	83.9	31.17	0.04	0.0	520.2	9.91
2	0.37	0.7	0.40	14.6	5.42	0.42	6.2	259.5	4.95
3	0.65	1.7	0.69	15.0	5.56	0.71	1.4	398.6	7.60
4	0.73	4.3	0.78	155.8	57.86	0.82	1.4	4068.9	77.54



Track 4, ID: WTEA

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	22.0	0.04	103.3	27.65	0.08	0.1	2613.3	24.02
2	0.29	9.7	0.30	14.1	3.78	0.32	1.1	200.3	1.84
3	0.41	17.6	0.46	64.3	17.22	0.52	2.8	2927.8	26.92
4	0.74	7.1	0.79	191.8	51.36	0.82	0.2	5136.2	47.22





DISCUSSION

HPTLC fingerprinting of phenolic compounds of the extracts of *WT* fruit and *DV* leaf at 254 nm in solvent system-acetic Acid: chloroform (1:9) showed the presence of 3 prominent peaks in DVC extract than DVM and same such peaks appeared in WTEA than the WTM at different Rf values indicating the presence of phenolics in WTEA and DVC. In solvent system B- ethyl acetate: benzene (9:11) showed good resolution and more number of phenolic compounds in DVC and WTEA extracts, Where as in solvent system- ethyl acetate: methanol: water (80:40:8) showed the presence of 3 phenolic compounds in both the ethyl acetate and methanolic extracts of *W. tinctoria* whereas 6 and 5 compounds in methanolic and chloroform extracts of *D.viscosa* respectively.

The results indicate the presence of phenolic compounds steroids and triterpenoids. Among various mobile phases tested the ethyl acetate: methanol: water (80:40:8) SOL-C, acetic acid: chloroform (1:9)-SOL-A and ethyl acetate: benzene (9:11) SOL-B shown better resolution of compounds in both the extracts. So from the observations both the extracts showed the presence of steroids/triterpenoids and phenolic compounds and further the presence of these compounds were confirmed by HPTLC studies.

CONCLUSION

Keeping in view the presence of phenolics, steroids and terpenoids in these plants, an attempt can be made to estimate the scavenging property of these plant extracts on free radicals thus can put check on various diseases. The Other methods of HPTLC analysis Like quantification of marker compounds from the extract, checking the quality of extracts, and detection of the different class of compounds from the extracts can be done.

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