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HPTLC ANALYSIS OF *DOLICHANDRONE ATROVIRENS* (SPRAGUE) PLANT BARK

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ABSTRACT

Dolichandrone atrovirens Sprague is a deciduous tree, It is belongs to the family Bignoniaceae. In this prospective study to evaluate the chromatogram detection of methanol extract of *Dolichandrone atrovirens* plant bark with standard flavonoid markers such as Apigenin, Rutin, Quercetin, Gallic acid and Catachin by HPTLC techniques. HPTLC Chromatogram was developed in methanolic extract *Dolichandrone atrovirens* plant bark and standard flavonoid marker compounds by using Toluene- Ethyl acetate- Formic acid- Methanol (3 : 6 : 1.6: 0.4) as mobile phase or solvent system. The identity of the bands of compounds 1-7 in the methanol extracts of *Dolichandrone atrovirens* were confirmed by overlaying their UV absorption spectra with the standard marker apigenin, rutin, quercetin, galic acid and catechin at 366 nm.

Key- Words: HPTLC, *Dolichandrone atrovirens*, UV absorption spectra, apigenin, rutin.

INTRODUCTION

India has a cultural rich heritage of traditional medicine comprised of two widely flourishing systems of Ayuvedic and Unani systems, since ancient times. The multiple therapeutic actions and used of crude herbal drugs are sufficiently described in classical literature on Indigenous medicines in many medicinal plant books and pharmacopoeias(1).

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. An important source of natural products, plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids[(2)].

Herbal drugs have been in use by different civilizations in different parts of the World for centuries to fight a large number of diseases. Many of these are in common use even today. However as herbal drugs are derived from heterogeneous sources leading to variations, which makes the standardization more important, as erroneous results can cause variations in phytochemical and pharmacological studies. The pharmacognostic characters, physicochemical values and results from phytochemical analysis could be used as a diagnostic tool for the standardization of medicinal plants used in herbal medicines[(3)].

The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards[(4)]. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters, hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations[(4&5)]. HPTLC fingerprinting technique offers better resolution and estimation of active constituents which can be done with reasonable accuracy in a shorter time[(6)].

Analytical separation techniques, like High performance liquid chromatography(HPLC), Gas chromatography(GC) and Mass spectrometry(MS) were among the most popular methods of choice used for quality control of raw material and finished herbal product. Finger print analysis approach using high performance thin layer chromatography(HPTLC) has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drugs[(7)].

The lack of pharmacological and clinical data on the majority of herbal medicinal products is medicinal practice. For valid integration, pharmacological studies must be conducted on those plants lacking such data[(8&9)]. A majority of the rich diversity of Indian medicinal plants

is yet to be scientifically evaluated for such properties[10]. The list of many such undocumented, unexploited and uncharacterized plants of medical importance have been brought to light by many researcher[11]. With this background, the present study to evaluate the chromatogram detection in one such unexplored plant is *Dolichandrone atrvirens* plant bark methanol extract with standard flavonoid markers such as Apigenin, Rutin, Quercetin, Gallic acid and Catachin by HPTLC techniques. *Dolichandrone atrovirens* plant bark was collected from Boda hills, Rasipuram taluk, Namakkal district, Tamilnadu, India. This plant bark was used for cancer treatment by Malayali tribes in Boda hills.

MATERIALS & METHOD

Extract preparation for HPTLC analysis

An aliquots of 100mg of methanol extract of *Dolichandrone atrovirens* was weighed in an electronic balance (Afcoset) and methanol extract dissolved in 1 ml of methanol. The solution was centrifuged at 3000rpm for 5min. The Supernatant was used as test solution for HPTLC analysis.

Samples Loading

2µl of the above test solutions and 2µl of standard solutions such as Apigenin, Rutin, Quercetin, Gallic acid and Catachin were loaded as 6mm band length in the 10 x 10cm Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Mobile phase

The organic solvents such as Toluene- Ethyl acetate- Formic acid- Methanol (3: 6: 1.6: 0.4) was used as a mobile phase. The twin trough developing chamber was saturated with the mobile phase for 10 minutes before the development of samples loaded TLC plate.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber with respective mobile phase (Flavonoid) and the plate was developed in the respective mobile phase up to 85mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV366nm.

Scanning

The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366nm. The Peak table, Peak display and Peak densitogram were noted.

RESULTS & DISCUSSION

Chromatogram was developed in *Dolichandrone atrovirens* methanol extract of sampe and standard flavonoid marker compound as apigenin, rutin, quercetin, galic acid and catechin under chamber saturation conditions using Toluene- Ethyl acetate- Formic acid- Methanol (3 : 6 : 1.6: 0.4) as mobile phase or solvent system. The identity of the bands of compounds 1-7 in the methanol extracts were confirmed by overlaying their UV absorption spectra with the standard marker apigenin, rutin, quercetin, galic acid and catechin at 366 nm. The methanol extract sample R_f value 0.81 and 0.72 was matches with flavonoids standard marker compound as apigenin, rutin, quercetin, galic acid and catechin. The methanol extract of *Dolichandrone atrovirens* plant bark showed the apigenin, rutin, quercetion, galic acid and catechin bands are identified and confirmed by comparing the chromatogram obtained from the reference standard solution (Figure 1-6 and Table 1-7) and comparing retention factor (Rf) value of apigenin, rutin, quercetin, galic acid and catechin from sample and standard solution.

HPTLC is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The presence or absence of chemical constituent has been found useful in the placement of plant in taxonomic categories. HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used for further in authentication and characterization of the medicinally important plant[(12)].

The HPTLC method can be used for phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to traditional system of medicine throughout the world[(13)]. The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such “database” for further mutifaceal sustainable studies.

HPTLC finger print analysis has become the most of its simplicity and reliability. It can serve as a tool for identification, authentication, qualitative, quantitative analysis and quality control of herbal drug[14,15&16].

Authentication of medicinal plants as genetic and chemical level is a critical step in the use of these botanical materials for both research purposes and commercial preparations. For any living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. In plant taxonomy, during this molecular era, the morphological characters also play a vital role in plant systematic study and are used as a tool for the classification of a taxon. In recent times, in addition to morphological markers, anatomical, cytological, biochemical and molecular markers are also being used to classify the organisms. HPTLC is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant. The developed HPTLC fingerprints will help the manufacture for quality control and standardization of herbal formulations. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies[17-22].

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials, and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved[23].

Table No. 1: Apigenin Track

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	0.6	0.01	38.9	7.10	0.03	7.0	598.6	4.78
2	0.16	15.6	0.19	28.3	5.17	0.20	25.2	541.0	4.32
3	0.21	30.0	0.24	40.9	7.47	0.31	1.4	1508.9	12.04
4	0.33	0.2	0.37	12.7	2.32	0.38	11.8	281.8	2.25
5	0.49	7.0	0.52	16.4	3.00	0.55	3.8	367.8	2.93
6	0.76	5.5	0.82	140.6	74.95	0.92	10.1	9234.9	73.68

Table No. 2: Rutin Track

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	14.1	0.01	56.1	15.22	0.02	2.3	486.1	5.97
2	0.06	0.4	0.08	12.6	3.41	0.09	8.9	144.7	1.78
3	0.09	9.5	0.14	269.8	73.14	0.20	15.2	6553.0	80.53
4	0.65	3.3	0.69	10.6	2.86	0.69	8.9	186.6	2.29
5	0.78	8.3	0.81	19.8	5.37	0.86	9.4	766.9	9.42

Table No. 3: *Dolichandrone atrovirens* Methanol extract Track

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	81.9	-0.00	106.7	20.44	0.01	1.2	721.0	4.51
2	0.10	0.3	0.17	61.6	11.79	0.19	37.4	2006.1	12.56
3	0.20	38.1	0.21	51.0	9.76	0.27	0.8	1433.1	8.97
4	0.35	4.1	0.38	12.2	2.34	0.41	2.8	279.3	1.75
5	0.59	3.0	0.72	182.6	34.96	0.78	78.9	8531.9	53.41
6	0.79	83.0	0.81	91.8	17.58	0.86	21.1	2700.9	16.91
7	0.88	14.6	0.89	16.4	3.13	0.92	6.7	302.4	1.89

Table No. 4: Quercetin Track

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	5.1	0.14	82.7	52.52	0.17	8.1	2616.7	55.94
2	0.76	23.8	0.81	74.8	47.48	0.85	13.0	2061.3	44.06

Table No. 5: Gallic acid Track

1	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.63	2.4	0.70	93.3	100.00	0.76	0.3	2493.7	100.00

Table No. 6: Catechin Track

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	5.9	0.14	15.5	16.66	0.15	10.4	523.2	20.16
2	0.61	3.6	0.65	13.1	14.15	0.66	11.1	297.3	11.45
3	0.68	11.3	0.73	64.2	69.20	0.77	8.5	1775.2	68.39

Table No. 7: HPTLC analysis on *Dolichandrone atrovirens* plant bark extracts correlate with standard flavonoids marker compound as Apigenin, Rutin, Quercetin, Galic acid and Catechin.

S. No	SAMPLES/ MARKER	Rf	HEIGHT	AREA
1	Apigenin	0.82	410.6	9234.9
2	Rutin	0.81	19.8	766.9
3	*Dolichandrone atrovirens* methanol bark extract	0.72	182.6	8531.9
		0.81	91.8	2700.9
4	Quercetin	0.81	74.8	44.06
5	Galic acid	0.70	93.3	2493.7
6	Catechin	0.73	64.2	1775.2

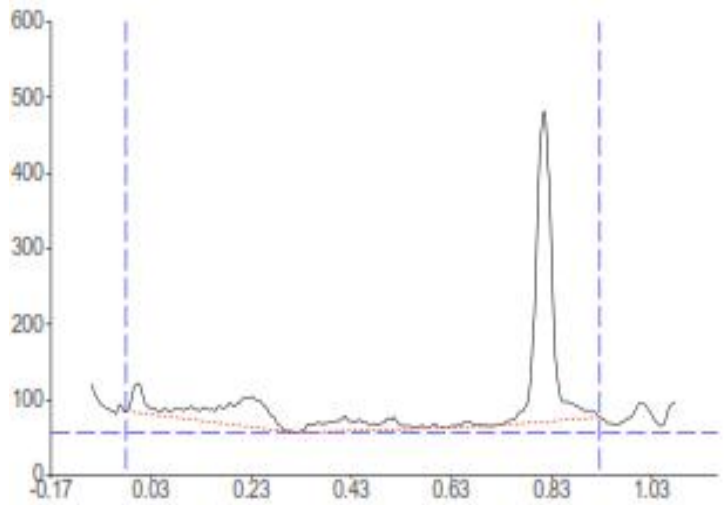

Fig. No. 1: Apigenin Chromatogram

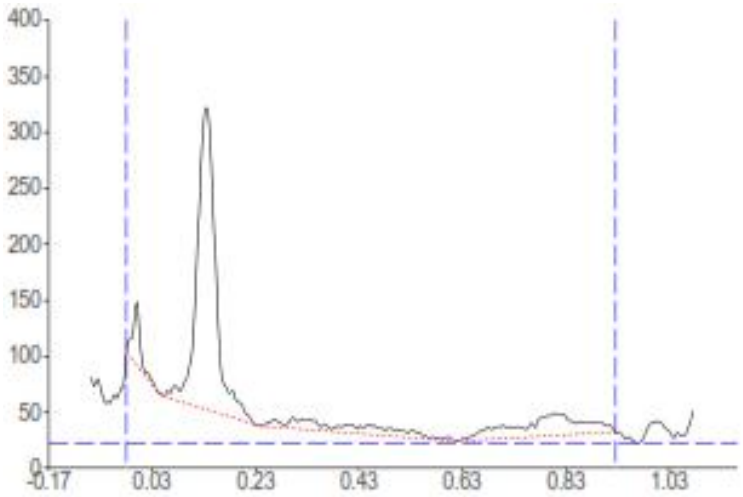

Fig. No. 2: Rutin Chromatogram

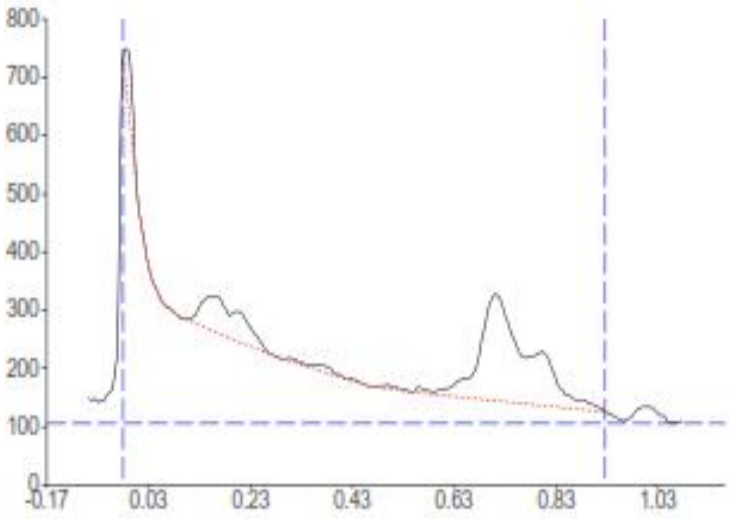

Fig. No. 3: *Dolichandrone atrovirens* Methanol extract Chromatogram

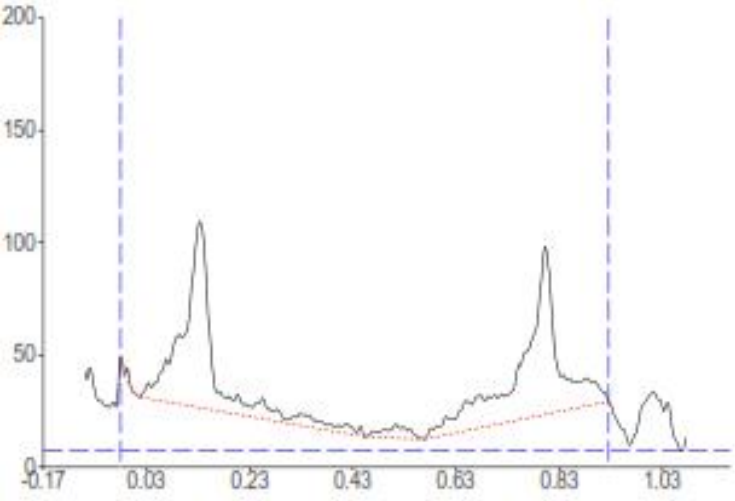

Fig. No. 4: Quercetin Chromatogram

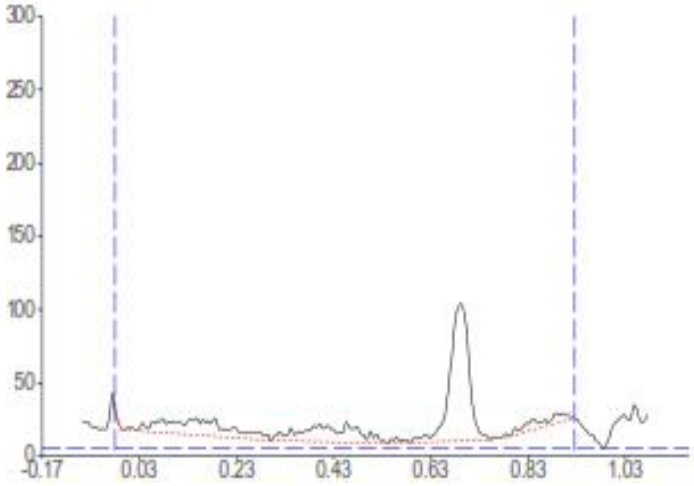

Fig. No. 5: Gallic acid Chromatogram

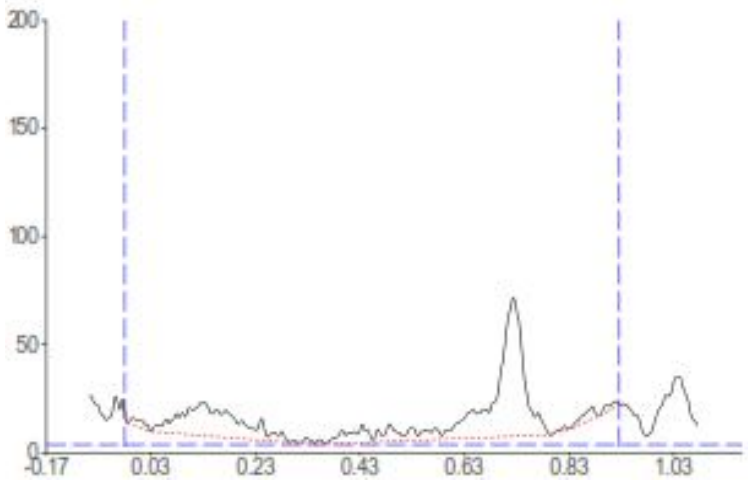

Fig. No. 6: Catechin Chromatogram

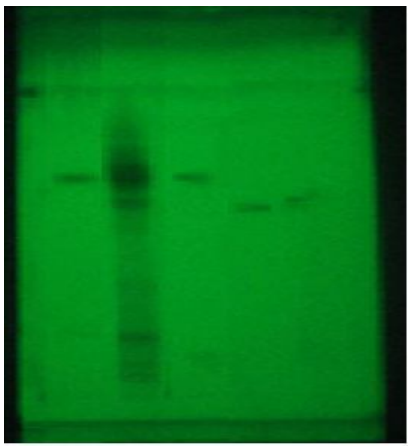

Fig. No. 7: HPTLC Chromatogram of Methanol extract of *Dolichandrone atrovirens* and Standard Flavonoids

CONCLUSION

The present study, the methanol extract of *Dolichandrone atrovirens* plant bark gave the evidence of the apigenin, rutin, quercetion, galic acid and catechin bands are identified and confirmed by comparing the chromatogram obtained from the reference standard flavonoid markers.

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