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Chromatographic fingerprint analysis of Leaf extracts of *Vitex leucoxylon* Linn by HPTLC technique.

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Research Article

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Abstract

To develop the finger print of medicinally and economically important leaves of *Vitex leucoxylon* Linn. Chromatographic techniques were used for separation of components from various extracts of dried leaves of *Vitex leucoxylon* Linn. in different solvents such as Petroleum ether, Benzene and Ethyl acetate. CAMAG HPTLC system equipped with Linomat V applicator, TLC Scanner 3, Reprostar 3 and WIN CATS -4 software were used. HPTLC finger printing of Petroleum ether extract of leaf revealed 8 peaks under 254 nm with Rf values in the range of
-0.02 to 0.86 and 5 peaks under 366 nm with Rf values in the range of -0.02 to 0.86; Benzene extract of leaf revealed 10 peaks under 254 nm with Rf values in the range of -0.03 to 1.10 and 8 peaks under 366 nm with Rf values in the range of 0.03 to 0.93; Ethyl acetate extract of leaf revealed 9 peaks under 254 nm with Rf values in the range of -0.03 to 0.91 and 8 peaks under 366 nm with Rf values in the range of -0.03 to 0.79. It can be concluded that HPTLC finger printing analysis of leaf extracts of *Vitex leucoxylon* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Keywords: *Vitex leucoxylon* Linn., Standardization, HPTLC finger printing, Absorption spectra.

Introduction

Plants have long provided mankind with medicine, with natural products once serving as the source of all drugs[1]. The rural population of the country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost[2]. According to World Health Organization (WHO) more than 80% of the world's population, mostly in developing countries depend on traditional plant based medicines for their primary healthcare needs[3]. India has about 45,000 plant species and among them many have been claimed to possess medicinal properties. The need for scientific validation of these useful medicinal plants are very essential[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 herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled technique and applying suitable standards[5]. Currently HPTLC is often used as an alternative to HPLC for the quantification of plant products because of its simplicity, accuracy, cost-effectiveness and rapidity[6]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time[7].

Vitex Leucoxylon Linn (Verbenreae) is a large deciduous tree, commonly known as Songarbhi (Marathi) an excellent herbal crude drug in the nature which has composition of the entire essential constituents that are required for normal and good health of human. It is small to large tree with a sort of thick trunk and a spreading crown and almost throughout the Deccan peninsula of India up to an altitude 900 meters, it extends northwards up to Jhansi and part of Bihar. The trees are generally found on the river bank, stream and ponds. The roots and the bark are astringent and roots are used as a febrifuge. The leaves are smoked for reliving headache and catarrh and are also used for medicinal baths and anaemia[8].

General pharmacological studies revealed anti-psychotic, anti-depressant, analgesic, anti-

inflammatory, anti-parkinsonian and anti-microbial activities of aqueous and ethanolic extracts of leaves of *Vitex Leucoxylon*[9,10] have studied the anti-inflammatory and wound healing properties of the crude alcoholic extract of the leaves in acute inflammation model[11]. The roots and bark are astringent and the roots are reported to be used as a febrifuge. β- sitosterol, dimethyl terphthalate, vitexin, isovitexin, agnuside and aucubin were isolated from the leaves or barks of *Vitex Leucoxylon*[12]. In this present study the HPTLC fingerprinting of *Vitex leucoxylon* leaf extract has been performed which may be used as markers for quality evaluation and standardization of the drug.

Material and Method

Selection of the plant material

The medicinal properties of plant have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities. The plant *Vitex leucoxylon* Linn leaves was selected for pharmacognostical standardization and HPTLC finger printing of its extracts.

Collection and authentication of plant material

The leaves of selected plant were collected in the month of April 2011 from ABS botanical garden Salem, Tamil nadu. The plant material was taxonomically identified by the Botanical survey of India, Coimbatore [voucher specimen no. BSI/SRC/5/23/2011-12] has been deposited in the museum of the department of Pharmacognosy, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Tamil nadu, India.

Preparation and extraction of plant material

The leaves of *Vitex leucoxylon* were dried under shade and powdered coarsely with a mechanical grinder and 500g was packed in a soxhlet apparatus and extracted successively with Petroleum ether, Benzene and Ethyl acetate. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by Whattman filter paper (no.1). The extract was evaporated under reduced pressure using Rotovac evaporator. The phytochemical investigation of the different leaf extracts of *Vitex leucoxylon* was carried out with standard protocol[13].

HPTLC finger print profile:

HPTLC studies were carried out following the method of Harborne[14] and Wager[15] *et al*.

Sample preparation:

The various leaf extracts were dissolved in HPTLC grade methanol which was used for sample application on pre-coated silica gel 60F254 aluminum sheets.

Developing solvent system

A number of solvent systems were tried for extracts, but satisfactory resolution was obtained in the following solvent.

Table 1 Ratio of mobile phase with Extracts

Name of the Extract	Mobile phase	Ratio
Petroleum ether	Hexane : ethyl acetate : formic acid	4.5 : 5 : 0.03
Benzene	Hexane : ethyl acetate : formic acid	4.5 : 5 : 0.03
Ethyl acetate	Hexane : ethyl acetate : IPA : formic acid	0.5 : 4 : 0.5 : 0.03

Sample application:

The samples (5, 10, 15, 20µl) were spotted in the form of bands of length 6.0 mm with a hundred micro liter samples using a Hamilton syringe on silica gel which was pre coated on aluminum plate 60F254 with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of chromatogram:

The mobile phase of chloroform extract consisted of hexane: ethyl acetate: formic acid (4.5:5:0.03) and 10ml of mobile phase was used per chromatography run. The linear ascending development was carried out in a (20cmx10cm) twin through glass chamber saturated with the mobile phase.

Detection of spots:

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 under UV light at 254 and 366nm respectively. The R_f values and finger print data were recorded by WIN CATS software.

Results and Discussion

The various extract of the plant of *Vitex leucoxylon* Linn. were subjected to phytochemical screening which reveals the presence of various pharmacological active compounds such as carbohydrates, protein and amino acids, tannins, flavonoids, phenolic compounds, saponins and glycosides are detected in *Vitex leucoxylon* various leaf extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs for human use.

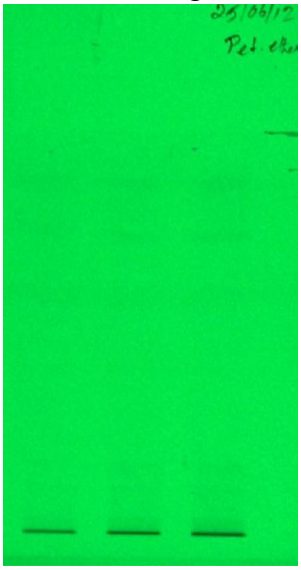
In this study the HPTLC fingerprinting of various extracts such as chloroform, ethanol and aqueous revealed several peaks under UV 366 and 254 nm was recorded in the corresponding figures.

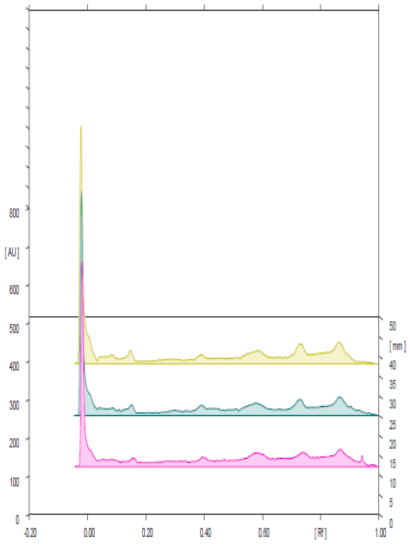
HPTLC fingerprinting of petroleum ether extract revealed 8 peaks under UV 254 nm (Figure 1& Table 2) at the following Rf value -0.04, 0.03, 0.06, 0.11,

0.36, 0.52, 0.70 & 0.83 and 5 peaks under UV 366 nm (Figure 2 & Table 3) at the following Rf value -0.04, 0.00, 0.07, 0.49,0.84. HPTLC fingerprinting of Benzene extract revealed 10 peaks under UV 254 nm (Figure 3& Table 4) at the following Rf value -0.04, 0.00, 0.15, 0.23, 0.29, 0.65, 0.77, 0.85, 0.97, 1.08 and 8 peaks under UV 366 nm (Figure 4 & Table 5) at the following Rf value 0.00, 0.16, 0.25, 0.31, 0.55, 0.62, 0.76, 0.91. HPTLC fingerprinting of Ethyl acetate extract revealed 9 peaks under UV 254 nm (Figure 5 & Table 6) at the following Rf value -0.04, -0.02, 0.17, 0.33, 0.42, 0.56, 0.65, 0.74, 0.91 and 8 peaks under UV 366 nm (Figure 6 & Table 7) at the following Rf value -0.04, -0.02, 0.09, 0.17, 0.32, 0.37, 0.57, 0.76.

PETROLEUM ETHER EXTRACT

HPTLC Chromatogram -254 nm **Spectra Scanned at 254 nm**

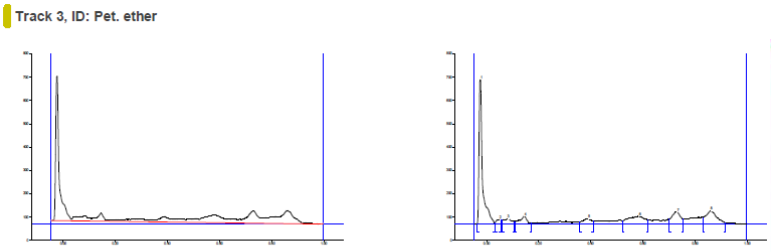




5 10 15 µl

Petroleum ether extract of *Vitex leucoxylon* under 254nm

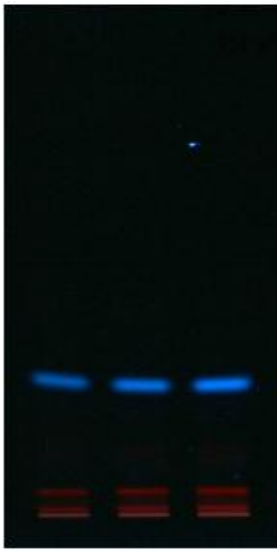
Table 2 HPTLC Profile of Petroleum ether extract of *Vitex leucoxylon* under 254nm

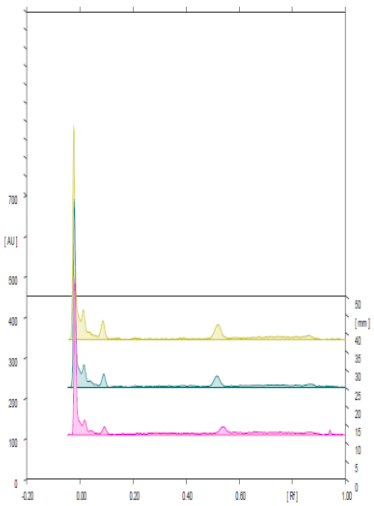


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	1.9	-0.02	620.2	71.43	0.03	7.5	6461.4	46.32	unknown *
2	0.03	8.3	0.05	19.6	2.26	0.06	18.5	274.3	1.97	unknown *
3	0.06	18.3	0.08	23.6	2.72	0.11	11.7	608.6	4.36	unknown *
4	0.11	11.9	0.15	35.1	4.05	0.17	6.6	783.2	5.61	unknown *
5	0.36	11.0	0.39	24.6	2.84	0.41	14.4	634.3	4.55	unknown *
6	0.52	14.0	0.59	35.4	4.08	0.62	18.0	1768.8	12.68	unknown *
7	0.70	24.2	0.73	52.2	6.01	0.76	24.2	1416.2	10.15	unknown *
8	0.83	30.1	0.86	57.4	6.61	0.92	6.7	2003.4	14.36	unknown *

PETROLEUM ETHER EXTRACT

HPTLC Chromatogram -366 nm **Spectra Scanned at 366 nm**

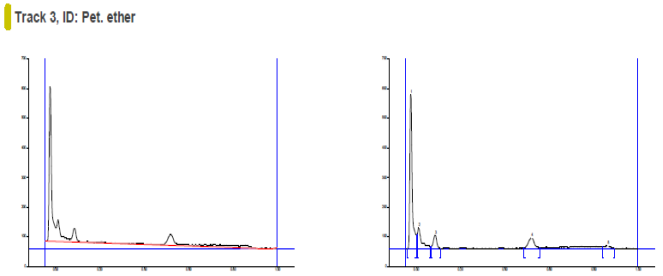




5 10 15 µl

Figure 2 HPTLC ProfilePetroleum ether extract of *Vitex leucoxylon* under 366nm

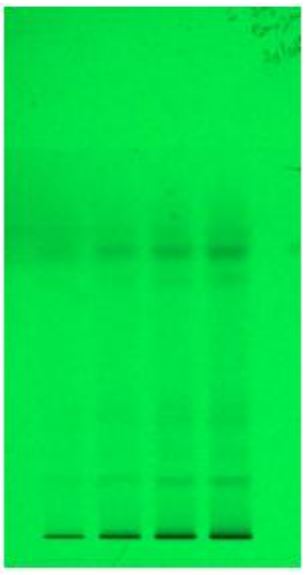
Table 3 HPTLC Profile of Petroleum ether extract of *Vitex leucoxylon* under 366nm

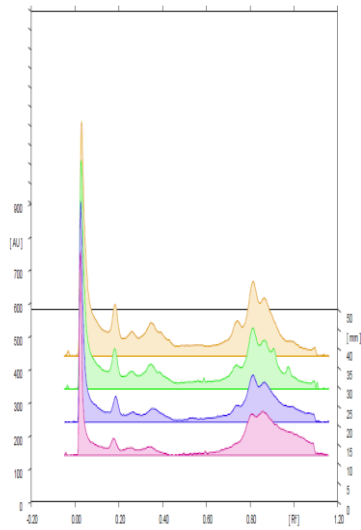


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	0.4	-0.02	522.7	75.71	0.00	46.1	4365.1	60.98	unknown *
2	0.00	47.5	0.01	73.1	10.59	0.06	8.3	1113.9	15.56	unknown *
3	0.07	8.6	0.09	46.7	6.77	0.11	1.5	598.0	8.35	unknown *
4	0.49	2.9	0.52	37.1	5.38	0.56	3.1	818.9	11.44	unknown *
5	0.84	7.3	0.86	10.8	1.56	0.90	2.1	262.1	3.66	unknown *

BENZENE EXTRACT

HPTLC Chromatogram - 254 nm **Spectra Scanned at 254 nm**

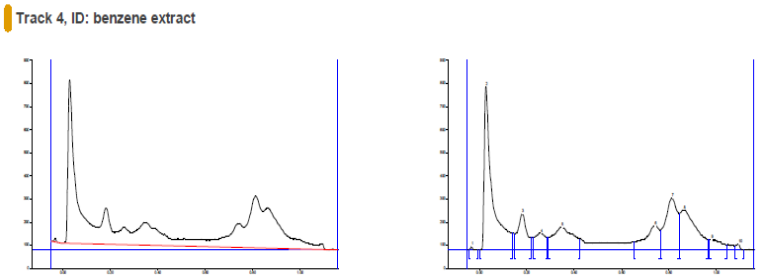




5 10 15 20 µ µµ

Figure 3 HPTLC Profile of Benzene extract of *Vitex leucoxylon* under 254nm

Table 4 HPTLC Profile of benzene extract of *Vitex leucoxylon* under 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	2.8	-0.03	15.3	0.94	-0.00	0.6	111.8	0.21	unknown *
2	0.00	0.3	0.03	708.1	43.35	0.14	74.4	16818.5	31.74	unknown *
3	0.15	71.4	0.18	155.8	9.54	0.22	52.8	4270.5	8.06	unknown *
4	0.23	54.2	0.26	74.3	4.55	0.29	53.9	2365.0	4.46	unknown *
5	0.29	55.0	0.35	100.6	6.16	0.43	48.6	6123.8	11.56	unknown *
6	0.65	34.5	0.74	105.7	6.47	0.76	86.9	4647.1	8.77	unknown *
7	0.77	87.5	0.81	225.4	13.80	0.85	159.5	8214.3	15.50	unknown *
8	0.85	159.7	0.86	175.5	10.74	0.97	45.6	8431.3	15.91	unknown *
9	0.97	44.2	0.98	46.5	2.85	1.05	22.3	1670.3	3.15	unknown *
10	1.08	18.4	1.10	26.1	1.60	1.12	0.6	334.7	0.63	unknown *

BENZENE EXTRACT

HPTLC Chromatogram - 366 nm **Spectra Scanned at 366 nm**

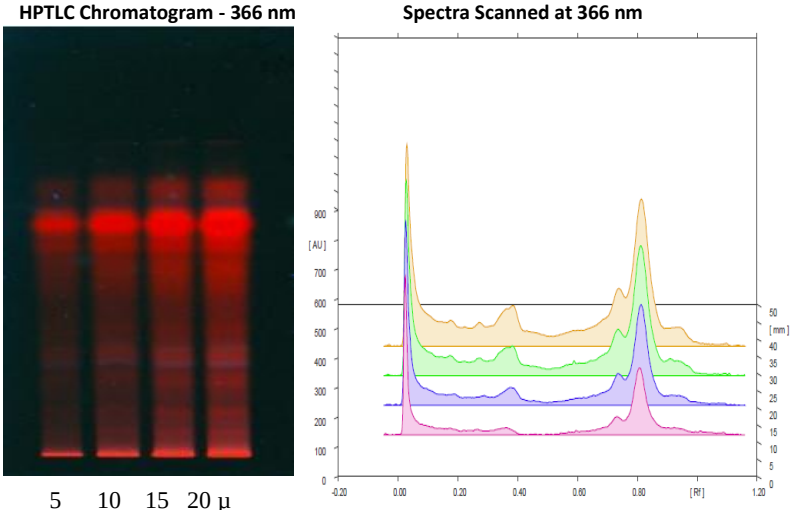


Figure 4 HPTLC Profile of Benzene extract of *Vitex leucoxylon* under 366nm

Table 5 HPTLC Profile of Benzene extract of *Vitex leucoxylon* under 366nm

ETHYLACETATE EXTRACT

HPTLC Chromatogram - 254 nm **Spectra Scanned at 254 nm**

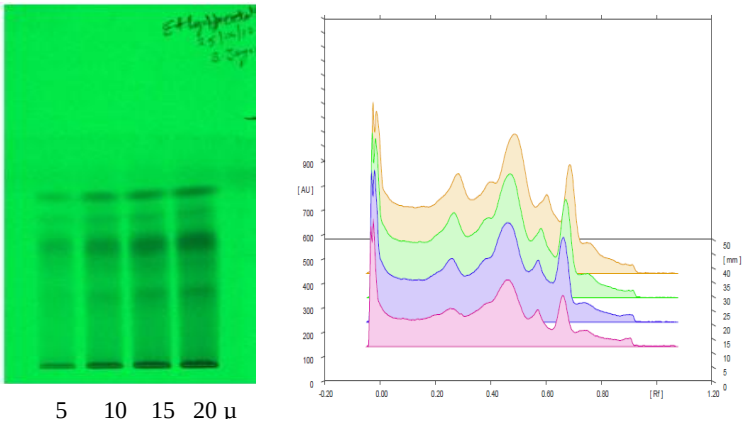
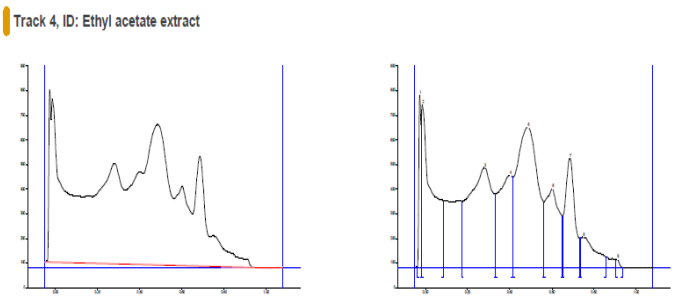


Figure 5 HPTLC Profile of Ethyl acetate extract of *Vitex leucoxylon* under 254nm

Table 6 HPTLC Profile of Ethyl acetate extract of *Vitex leucoxylon* under 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	5.5	-0.03	702.3	19.21	-0.02	608.2	5727.3	3.65	unknown *
2	-0.02	642.5	-0.01	664.9	18.19	0.09	274.1	23148.6	14.76	unknown *
3	0.17	271.6	0.28	408.7	11.18	0.33	302.1	32906.4	20.98	unknown *
4	0.33	302.2	0.40	376.3	10.29	0.42	370.9	17811.4	11.35	unknown *
5	0.42	370.9	0.49	571.3	15.63	0.56	271.1	40780.2	25.99	unknown *
6	0.56	271.4	0.60	324.2	8.87	0.65	211.5	15017.0	9.57	unknown *
7	0.65	211.9	0.69	446.3	12.21	0.73	122.5	14509.6	9.25	unknown *
8	0.74	122.8	0.75	125.7	3.44	0.86	44.8	6579.6	4.19	unknown *
9	0.91	33.6	0.91	36.1	0.99	0.94	2.6	401.1	0.26	unknown *

ETHYLACETATE EXTRACT

HPTLC Chromatogram - 366 nm

Spectra Scanned at 366 nm

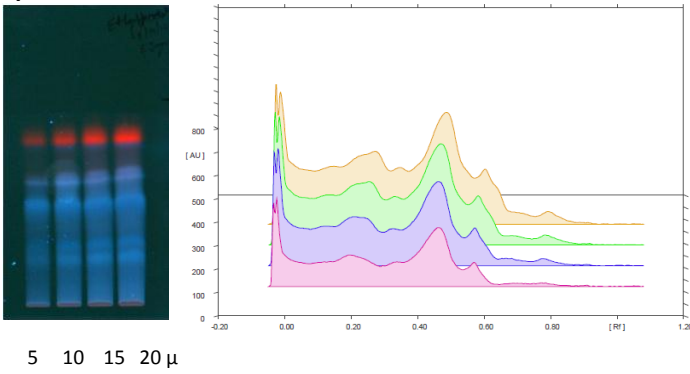
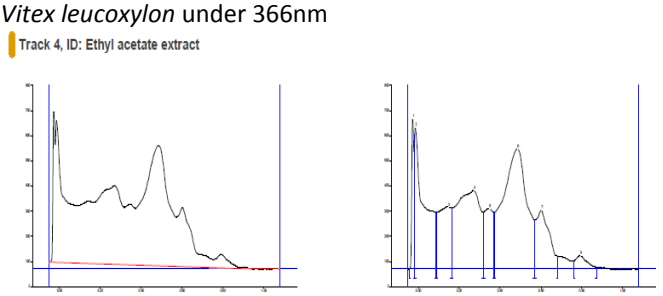


Figure 6 HPTLC Profile of Ethyl acetate extract of *Vitex leucoxylon* under 366nm

Table 7 HPTLC Profile of Ethyl acetate extract of *Vitex leucoxylon* under 366nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	1.7	-0.03	597.7	21.86	-0.02	498.4	5004.9	4.05	unknown *
2	-0.02	535.8	-0.01	564.8	20.66	0.09	227.1	19252.0	15.58	unknown *
3	0.09	227.2	0.15	247.5	9.05	0.16	244.4	11276.0	9.13	unknown *
4	0.17	244.5	0.27	312.9	11.45	0.32	228.0	26625.0	21.55	unknown *
5	0.32	228.3	0.35	242.1	8.86	0.37	225.6	7750.2	6.27	unknown *
6	0.37	225.6	0.49	478.5	17.50	0.57	197.4	40058.0	32.43	unknown *
7	0.57	197.4	0.60	235.5	8.62	0.69	51.9	11252.7	9.11	unknown *
8	0.76	34.6	0.79	54.6	2.00	0.87	7.6	2318.1	1.88	unknown *

Conclusion

Vitex Leucoxylon Linn is commonly being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Based on the traditional uses and the literature survey, the plant was selected for phytochemical evaluation and HPTLC finger print for various leaf extract of *Vitex leucoxylon* Linn. The preliminary phytochemical screening tests may be useful in the detection of bioactive principles.

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. Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixing standards to this plant.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.