Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 970-977
Received: 09-01-2019
Accepted: 11-02-2019

S Sujatha
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Dr. T Sekar
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Correspondence
S Sujatha
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Phytochemical screening and HPTLC method for phytochemical compounds present extracts of leaf and stem *Litsea laevigata* Gamble

S Sujatha and Dr. T Sekar

Abstract
Objective: In the present study, the phytochemical analysis determines *Litsea laevigata* leaf and stem extract used as non-polar to a polar solvent of pet-ether, chloroform, acetone methanolic and water. Leaf and stem investigated by applying established *in vitro* studies. L. laevigata belongs to the Lauraceae family.
Method: To confirm the fingerprint outline of *Litsea laevigata* using high-performance thin layer chromatography (HPTLC) procedure. Methods: Preliminary phytochemical screening was performed and HPTLC investigations were provided an explanation. CAMAG HPTLC system provided with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software was done.
Results: Preliminary phytochemical screening of the extract showed the presence of flavonoid, reducing suger, tannins, saponnins, phenolic compounds, steroid and terpenoid.
HPTLC finger printing of methanolic extract of leaf and stem revealed 3 and 9 peaks of phenol compound delectation, with Rf values start from 0.02 to 0.67 in leaf and 0.03 to 0.90 in stem. Flavonoid delectation of leaf and stem revealed 6 and 7 peaks with Rf values start from 0.02 to 0.99 in leaf and 0.05 to 0.99 stem.
Conclusions: It can be assumed of HPTLC fingerprint investigation of leaf and stem extract of Litsea laevigata can be done as a characteristic tool for the accurate identification of the plant and it is valuable as a phytochemical marker and also an immeasurable estimator of transmitted variability in plant communities.

Keywords: *Litsea laevigata*, phytochemical screening, HPTLC fingerprinting

Introduction
India has one of the richest and most diverse cultural traditions associated with the use of medicinal plants. The amount of phytochemical substances ranges considerably from species to species and indeed from plant to plant, depending on the age and various ecological and climatic factors. Plants have limitless ability to incorporate aromatic elements, mostly phenols or their oxygen-substituted derivatives (Geisssman 1963) [3]. Phytochemicals are advantageous to boost up immunology replies and also provide immunity to many diseases. Some phytochemicals are known to reveal medicinal and physiological activities which are phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids, phytosterols etc (Yadav 2017) [5].
The various prominent of certain bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological knowledge is frequently considered an adequate approach in the discovery of new anti-infective agents from a higher plant (Farnsworth 1966) [6]. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke metabolic syndrome (Singh 2003) [7]. Hot continuous extraction of Soxhlet is one of the techniques of medicinal plants extraction of secondary metabolites. Soxhlet extraction strongly depends on matrix characteristics and particle size as the internal diffusion may be the limiting step (Lijun Wang 2006) [8].
Medicinal herb is estimated to be a biochemical laboratory as it contains an abundance of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, lactones and essential oils (Amrit Pal Singh, 2005) [1]. Flavonoids are the combination of polyphenolic compounds which are several structure and properties. Plant phenols products of the phenylpropanoid pathway. Influential antioxidants and might limit oxidative destruction to biomolecules such as DNA, lipids, and proteins which play a role in chronic as cancer and cardiovascular disease (Cook NC 1996) [4].
High-performance thin layer chromatography (HPTLC) is a modern instrumental procedure based on the full abilities of thin layer chromatography.

The primary system of HPTLC applies for separation, determination and validation process. HPTLC would be a proper technique for resolving the difficulty because of its highly accurate data. HPTLC is the most simple separation technique accessible to the analyst (Shrivastava Manmohan 2010) [12]. HPTLC offers better resolution and estimation of active ingredients can be done with reasonable accuracy in a shorter time. HPTLC has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its purity, sensitivity, accuracy, suitability for high throughput screening, etc. (Sethis 1996) [13].

High-performance TLC (HPTLC) is an accomplished instrumental separation system, and optimized quantitative HPTLC practicing densitometric evaluation can provide effects comparable to those received with gas chromatography (GC) and high-performance liquid chromatography (HPLC) (Wagner 2001) [9]. The HPTLC is displaying a regular analytical system due to its benefits of low performing cost, high sample throughput and the requirement for least sample clean-up. An additional significant advantage of HPTLC is that various samples can be run concurrently using a minimum quantity of mobile phase, unlike HPLC, thus reducing analysis time and cost per analysis (Nile & Park 2014) [11].

Material and Methods

Plant material

The leaves part of *L. laevigata* was collected from Gudalur of Western Ghats, during April 2017. The collected plant material was identified and authenticated by Botanical Survey of India, Southern Circle, Coimbatore (NO.BSI/SRC/5/23/2017/Tech./17) and the voucher specimen has been deposited in Bharathiar University Herbarium, Department of Botany, and Coimbatore. The collected fresh plant for leaves was cleaned thoroughly with running tap water to remove dust and shade dried for a week at room temperature. The powders were in the airtight container.

Chemicals and reagents

Ferric chloride, α-naphthol, potassium persulfate, copper phosphate, hydrochloric acid, sulphuric acid, trichloroacetic acid, riboflavin, ethylenediaminetetraacetic acid (EDTA), potassium sodium tartrate, sodium hydroxide, sodium nitrite, aluminum chloride, potassium hydroxide, acetic anhydride, gallic acid, and quercetin. All other reagents used were of analytical grade.

The chemicals and elements were acquired from Sigma. Aldrich chemical company, St. Louis, U.S.A. All other chemicals and solvents were obtained from Himedia, SRL and SD fine chemicals Mumbai, India and were of the highest purity and analytical grade.

Plant extracts preparation

The powder plant material was extracted as one of the extraction method of Soxhlet extractor based extracted continuously with petroleum ether, chloroform, acetone, methanol, and water. Each time before extricating with the following dissolvable, the thimble was dried in hot air stove underneath 40ºC. The distinctive dissolvable concentrates were thought by rotating vacuum evaporator and after that air dried. The dried concentrate got with every dissolvable was weighed. The rate yield was communicated as far as the air-dried weight of plant material.

In vitro Phytochemical test

Carbohydrates (Molish test)

About 100mg of the extract was dissolved in 5 ml of water and filtered. Two drops of the alcoholic solution of α – naphthol was added to 2ml of filtrate and 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates [14].

Proteins (Biuret test)

The filtrate extract (100mg) was dismissed in 10ml of distilled water and separated through Whatman No.1 filter paper. The 2ml aliquot of the filtrate was treated with one drop of 2% copper sulfate solution. To this 1 ml of 95% ethanol was added, followed by an excess of potassium hydroxide pellets. The pink color in the ethanolic layer indicated the presence of proteins [14].

Amino acids (Ninhydrin test)

Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) was added to 2 m of aqueous filtrate. The presence of amino acids was indicated by the presence of a characteristic purple color [14].

Reducing Sugar (Fehling's test)

One mL of filtrate is boiled on a water bath with 1mL each of Fehling's solutions A and B, a Red precipitates the presence of sugar.

Fehling's solution A: Copper sulfate (2 g) is dissolved in distilled water and made up to 30 mL using distilled water. Fehling's solution B: Potassium sodium tartrate (10 g) and sodium hydroxide (3 g) is dissolved in water and made up to 30 mL.

Benedict's test

An equal volume (2ml each) of Benedict's solution and extracts were mixed in a test tube and heated in boiling water bath for 10min the changes in color to yellow, green and red indicates the presence of reducing sugars.

Phenolic compounds ($FeCl_3$ test)

The filtrate extract (50 mg) is added in 5mL of distilled water. To this, few drops 5% inactive ferric chloride solution was added. Phenolic compounds were indicated by the presence of dark green color [15].

Gum and mucilage test

The filtrate extract (100mg) is dissolved in 10ml of distilled water and to this, 25ml of absolute alcohol is added with constant stirring. While or cloudy precipitate indicates the presence of gums and mucilage.

Test for Tannins (Braymer's Test)

1ml of filtrate extract distilled water was added for reducing concentration after two drops of ferric chloride were added. A transient greenish to black color indicates the presence of Tannins [14].

Steroid and terpenoid

1. Salkowski test: Test the extract with 5ml chloroform and few drops of conc. sulphuric acid added to the side of the test tube wall, red color at lower layer indicate the presence of steroid and formation of yellow colored lower layer indicates the presence of triterpenoid [14].

2. Libermann-Burchard Test: In a test tube, a small quantity of the solid sample was added 5 mL of chloroform solution treated with a few drops of concentrated H2SO4 and 2 mL acetic anhydrides. Green color shows the presence of steroid and terpenoid type compounds [14].

Saponins test: (Form test)
The curde extract (50mg) is reduced with distilled water and made up to 20 mL. The filtrate is added to the grading cylinder for 15 min. A 2cm layer indicates the presence of saponins [14].

Flavonoids test
The curde extract becomes to be employed with a few drops of sodium hydroxide solution. The configuration of intense yellow color, which becomes colorless on the counting of acid, indicates the presence of flavonoids [16].

Alkaloids
Mayer's test
0.5 ml of filtrate and 2 drops of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the test as positive [17].

Wegner's test
0.5 ml of filtrate and 2 drops of Wegner reagent are added by the side of the test tube, a reddish- brown precipitate confirms the test as positive [17].

Dragendorff's test
0.5 ml of filtrate and 2 drops of Wegner reagent are added a prominent yellow precipitate indicates confirms the test as positive.

HPTLC Profile
Preparation of the Extract
35mg of the crude methanolic extract stem and leaf of *L. laevigata* were immersed in a mixture containing methanol (1:1) for 48 hours. The extract was stored in a separate airtight container.

Preparation of standard solution
Gallic acid used as the standard of to determination of phenol. 15.0µl of Gallic acid was applied to the HPTLC plate in the application position 85.0mm. Qucertein was the determination of flavonoid, used as for standard. 15.0µl quertein was applied the volume to the HPTLC plate and application position for 85.0mm.

Chromatographic condition
The chromatogram was developed on 10×20cm aluminum thin layer chromatography (TLC) plate precoated with 0.2mm layer of silica gel 60 F 245(Merck Ltd Germany) stored in a desiccator. The application was done by Camag Linomat syringe (100µl sec-1) mounted on a Linomat Vapplicator. Application of bands stem and leaf extract applied volume 15.0µl and position for leaf 15.0mm and stem 50mm for compared to phenol as the same applied volume and position of flavonoid, and its comparison to the quertein of with the help of Linomat V applicators attached to Camag HPTLC system which was programmed through win CATS software (Version 1.3.0) at 254nm and 366nm using Deuterium light source.

Development of mobile phase
The spotting was done on the TLC plate, ascending development of the plate, Camag TLC Scanner detection of scan start position the lower 10mm to 70mm higher was performed. Mobile phase I used for phenol analysis Chloroform-5ml, Ethyl Acetate-4ml, Formic Acid-1ml.
Flavonoid analysis was using mobile system II of the chloroform-8.5ml, methanol - 1ml, formic acid-0.5ml as. in a Camag chamber previously saturated with solvent vapour for 20 minutes. After development, the plate was dried at 60°C in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner III equipped with the win CATS Software.

Detection of spots
The chromatograms were scanned by the densitometer at 254nm 366nm and R white. The Rf values and fingerprint data were recorded and the plate was in photo documentation chamber and captured the images.

Results
Preliminary phytochemical analysis
Phytochemical screening of plant extract of *L. laevigata* showed the presence of various constituents viz. The qualitative phytochemical screening of leaf, the stem of *L. laevigata* for major primary and secondary photochemical are shown in table 1. The results revealed that the primary metabolites such as proteins, carbohydrate, and amino acids are present in the appreciable amount in all the parts. Among the stem and leaf are used to seem therapeutically important part which possesses a higher amount of secondary metabolites. The secondary metabolites such as phenolic compounds, flavonoids, tannins, saponins, steroid, and terpenoid were found to be variously distribution in leaf and stem of *L. laevigata*.

Table 1: Photochemical screening of various extract of *Litsea laevigata*

S.no	Phytochemical test	Pet ether		Chloroform		Acetone		Methanol		Water	
		L.L.L	L.L.S	L.L.L	L.L.S	L.L.L	L.L.S	L.L.L	L.L.S	L.L.L	L.L.S
1	**Alkaloid**										
	Mayer reagent	-	-	-	-	-	-	-	-	-	-
	Wager test	-	-	-	-	-	-	-	-	-	-
	Dragendroff's reagent	-	-	-	-	-	-	-	-	-	-
2	**Carbohydrates**										
	Molish test	+	+	+	+	+	+	+	+	+	+
3	**Protein and amino acid**										
	Biuret test	++	++	++	++	++	++	++	++	++	++
4	**Reducing sugar**										
	Fehling's test	++	++	++	++	++	++	++	++	++	++
	Benedict's reagent	++	++	++	++	++	++	++	++	++	++
5	**Flavonoid**										

	$FeCl_3$ test	+	+	+	+	++	++	++	++	++	++
6	**Tannin**	+	+	+	+	+	+	+	+	+	+
7	**Phenolic compound**	++	++	++	++	++	++	++	++	++	++
8	**Steroid and terpenoid**										
	Salkowski test	++	++	++	++	++	++	++	++	++	++
9	**Saponin test**										
	Frothing test	+	+	+	+	+	+	+	+	+	+
10	**Gum and mucilage**	+	+	+	+	++	++	++	++	++	++

(-) Absence of chemical compound, (+) minimum, (++) moderate level.

HPTLC

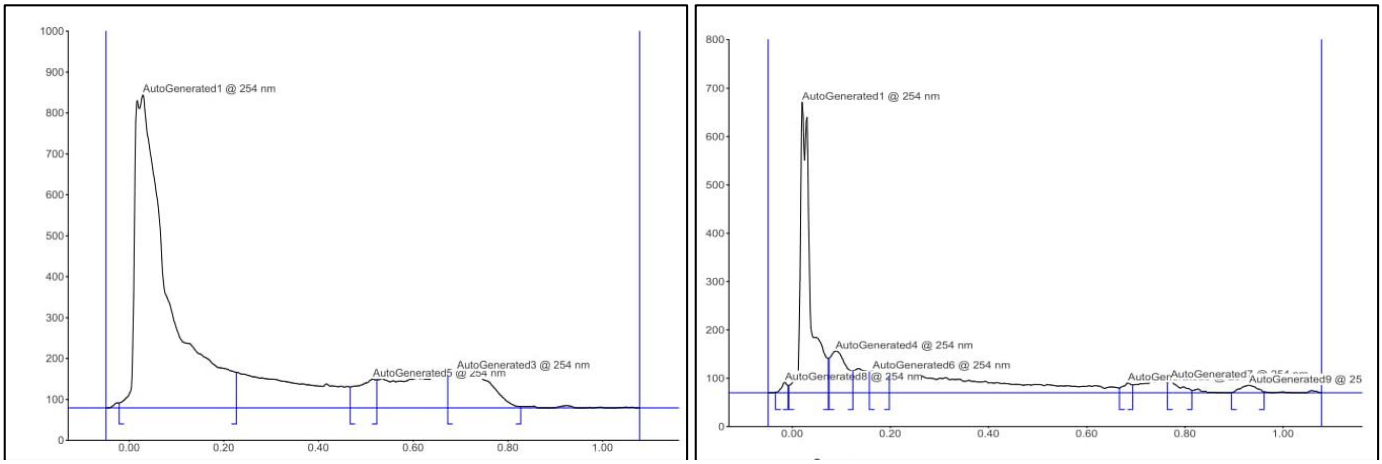
The results from HPTLC fingerprint scanned at different wavelengths for methanol extract of *L. laevigata* stem and leaf confirmed the presence of different secondary metabolites with different concentrations.

Phenols

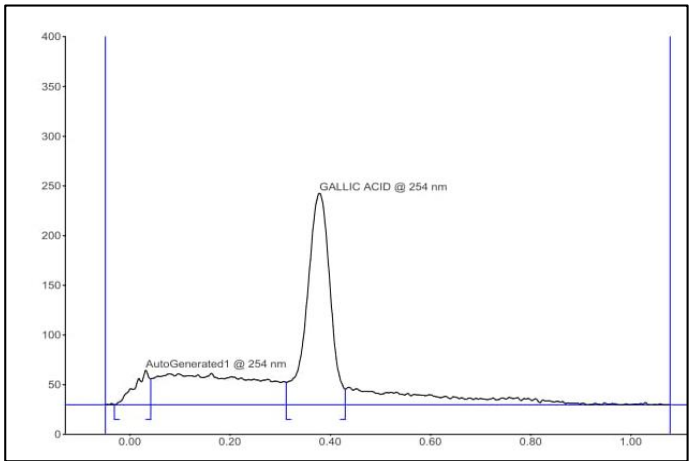
As shown in the figure 1, track 1 and 2 loaded with 5.0µl of methanol extract leaf and stem showed the presence of 3 and 9 peak phytoconstituents and corresponding ascending order of Rf values start from 0.02 to 0.67 in leaf and 0.03 to 0.90 stem. Which highest concentration of the phytoconstituents was found in leaf 83.52% and stem 66.78% whose corresponding Rf value was found leaf and stem recorded in Table 2 confirmed the presence of phenols in L. laevigata. Phenolic conformation tract 3 Gallic acid applied standard and Rf values start from 0.03 to 0.31. Which highest concentration of the phytoconstituents was found to be 90.20%.

Flavonoids

As shown in Figure 2, track 1 and 2 loaded with 5.0µl of methanol extract leaf and stem showed the presence of 6 and 9 peak phytoconstituents and corresponding ascending order of Rf values start from 0.02 to 0.99 in leaf and 0.05 to 0.99 stem. Which highest concentration of the phytoconstituents was found in leaf 43.17% and stem 14.94% whose corresponding Rf value was found leaf and stem recorded in table 3, as confirmed the presence of phenols in *L. laevigata*. Phenolic conformation tract 3 qucertein applied standard and Rf values start from 0.03 to 0.54. Which highest concentration of the phytoconstituents was found to be 72.67%.



A. B.



C.

Fig 1: A- HPTLC Chromatogram tract 1 (L.L. LEAF Methanol extract shows Phenol) at 254nm, B- HPTLC Chromatogram tract 2 (L.L. STEM Methanol extract shows Phenol) at 254nm, C-HPTLC Chromatogram Tract 3 (GALLIC ACID standard shows Phenol) at 254nm

Table 2: Rf value of the chromatogram of L.L. LEAF Methanol extract of phenol

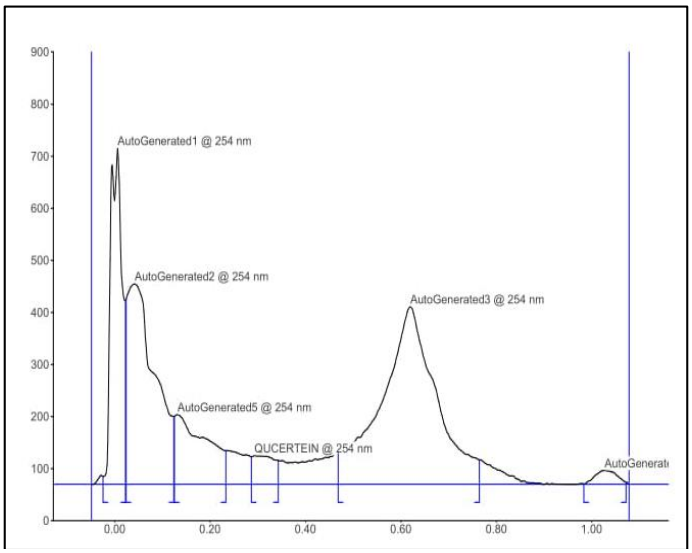
Peak	Start		Max			End		Area		Assigned subtance
	Rf	H	Rf	H	%	Rf	H	Area	%	
1	0.02	12.2	0.03	765.0	82.66	0.23	86.6	38584.2	83.52	Auto Generated 1
2	0.47	51.4	0.52	70.7	7.64	0.54	69.3	2162.8	4.68	Auto Generated 5
3	0.67	77.0	0.69	89.8	9.70	0.83	3.5	5448.2	11.79	Auto Generated 3

Table 3: Rf value of the chromatogram of L.L. STEM methanol extract of phenol

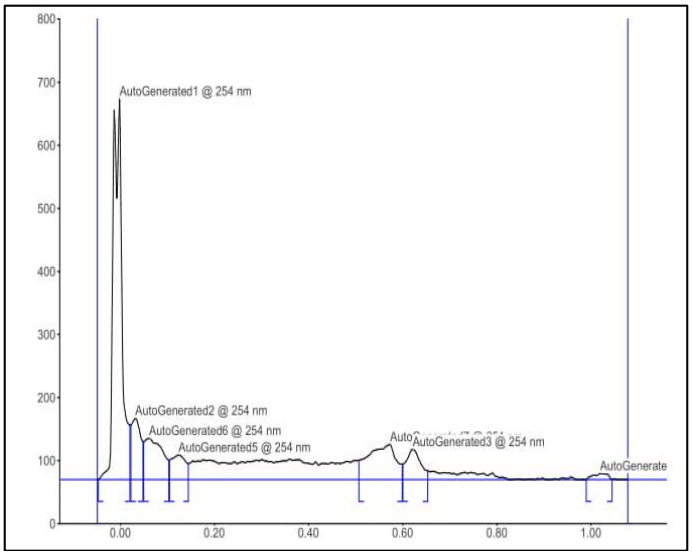
Peak	Start		Max			End		Area		Assigned subtance
	Rf	H	Rf	H	%	Rf	H	Area	%	
1	0.03	1.4	0.01	21.8	2.66	0.01	15.6	189.3	1.40	Auto Generated 8
2	0.01	15.6	0.02	601.0	73.46	0.07	71.3	9046.8	66.78	Auto Generated 1
3	0.08	72.0	0.09	86.7	10.60	0.12	45.4	2115.6	15.62	Auto Generated 4
4	0.16	44.0	0.16	45.7	5.59	0.20	34.6	1061.3	7.83	Auto Generated 6
5	0.67	9.8	0.69	20.5	2.51	0.70	16.6	283.2	2.09	Auto Generated 3
6	0.77	20.8	0.77	26.6	3.25	0.82	5.1	461.9	3.41	Auto Generated 7
7	0.90	0.9	0.93	15.9	1.94	0.96	2.1	389.1	2.87	Auto Generated 9

Table 4: Rf value of the chromatogram of GALLIC ACID standard of phenol

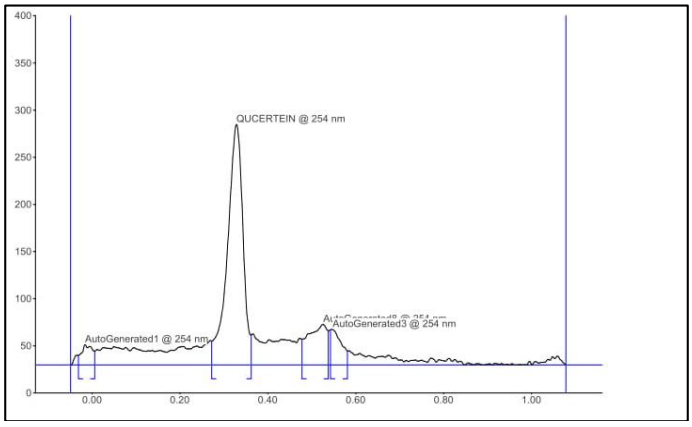
Peak	Start		Max			End		Area		Assigned subtance
	Rf	H	Rf	H	%	Rf	H	Area	%	
1	0.03	0.1	0.03	35.0	14.11	0.04	26.3	763.5	9.80	Auto Generated 1
2	0.31	22.8	0.38	213.0	85.89	0.43	15.8	7027.2	90.20	Gallic acid



A.



B.



C.

Fig 2A: HPTLC Chromatogram tract 1 (L.L. LEAF Methanol extract shows Flavonoid) at 254nm, B- HPTLC Chromatogram tract 2 (L.L. STEM Methanol extract shows Flavonoid) at 254nm, C-HPTLC Chromatogram Tract 3 (QUCERTEIN standard shows Flavonoid) at 254nm

Table 5: Rf value of the chromatogram of L.L. LEAF methanol extract of Flavonoid

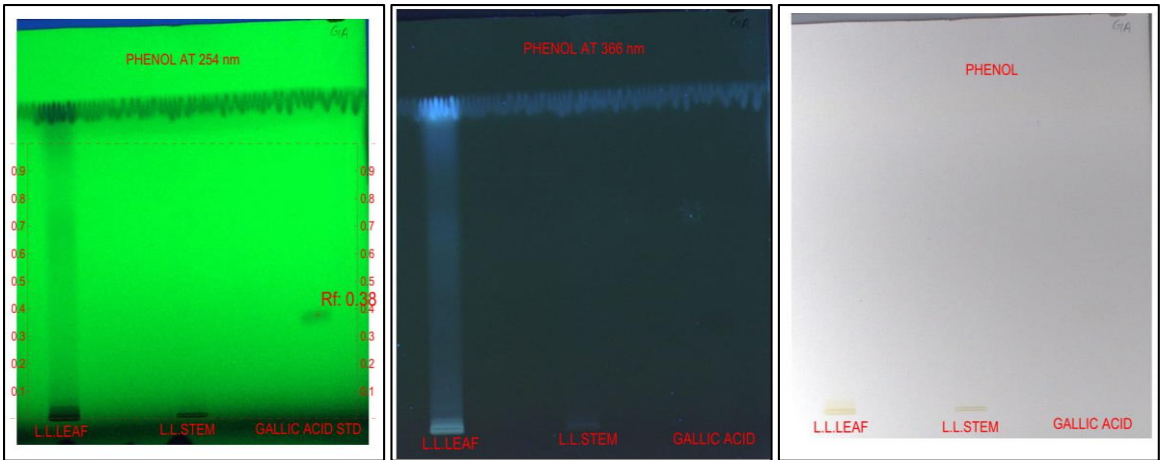
Peak	Start		Max			End		Area		Assigned subtance
	Rf	H	Rf	H	%	Rf	H	Area	%	
1	0.02	15.7	0.01	656.1	40.61	0.02	353.4	11354.4	17.47	Auto Generated 1
2	0.01	356.3	0.04	385.2	24.25	0.12	130.7	16348.4	25.16	Auto Generated 2
3	0.13	131.1	0.13	134.4	8.46	0.23	64.8	6436.7	9.90	Auto Generated 5
4	0.29	53.8	0.29	55.4	3.49	0.34	46.3	1883.9	2.90	QUCERTEIN
5	0.47	59.8	0.62	341.5	21.50	0.77	47.5	28053.8	43.17	Auto Generated 3
6	0.99	1.1	1.03	26.9	1.70	1.07	4.3	909.3	1.40	Auto Generated 9

Table 6: Rf value of the chromatogram of L.L. STEM methanol extract of Flavonoid

Peak	Start		Max			End		Area		Assigned subtance
	Rf	H	Rf	H	%	Rf	H	Area	%	
1	0.05	1.3	0.01	603.8	65.54	0.02	86.6	8186.0	51.13	Auto Generated 1
2	0.02	86.9	0.03	97.4	10.57	0.05	60.4	1421.1	8.88	Auto Generated 2
3	0.05	605	0.06	65.7	7.14	0.10	31.0	1823.5	11.39	Auto Generated 6
4	0.10	31.1	0.12	39.8	4.32	0.15	25.6	884.2	5.52	Auto Generated 5
5	0.51	31.3	0.57	55.9	6.07	0.60	24.9	2392.3	14.94	Auto Generated 7
6	0.60	25.1	0.62	48.3	5.24	0.65	14.1	1072.8	6.70	Auto Generated 3
7	0.99	0.5	1.02	10.3	1.12	1.05	0.8	229.8	1.44	Auto Generated 9

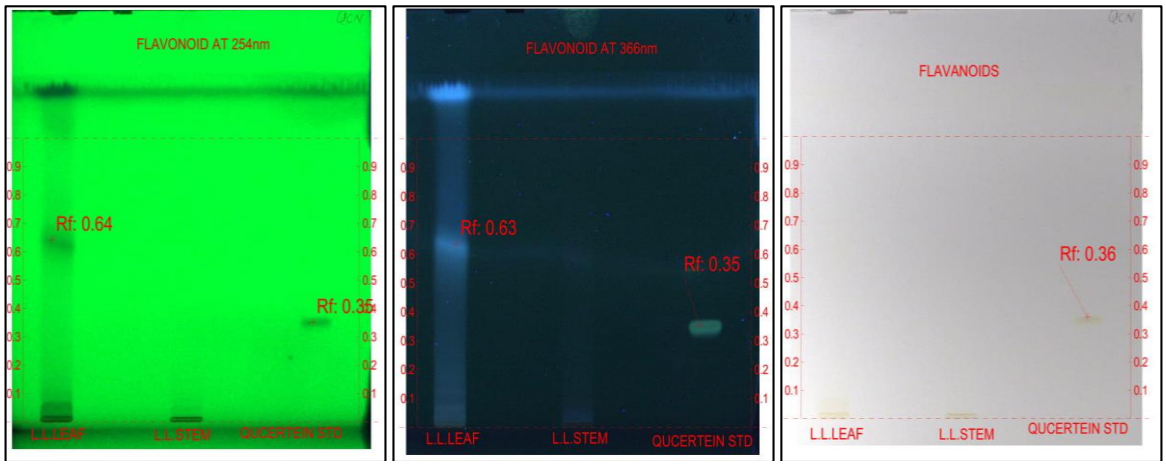
Table 7: Rf value of the chromatogram of Qucertein standard shows Flavonoid

Peak	Start		Max			End		Area		Assigned subtance
	Rf	H	Rf	H	%	Rf	H	Area	%	
1	0.03	10.2	0.02	21.7	6.05	0.01	14.5	395.8	4.45	Auto Generated 1
2	0.27	25.5	0.33	255.2	71.26	0.36	31.7	6470.2	72.67	Qucertein
3	0.48	27.5	0.53	43.4	12.12	0.54	36.2	1350.1	15.16	Auto Generated 8
4	0.54	37.4	0.55	37.9	10.57	0.58	14.6	687.5	7.72	Auto Generated 3



R-254nm R-366nm R-White

Fig 3: HPTLC profile of Phonol



R 254nm R 366nm R white

Fig 4: HPTLC profile of Flavonoid

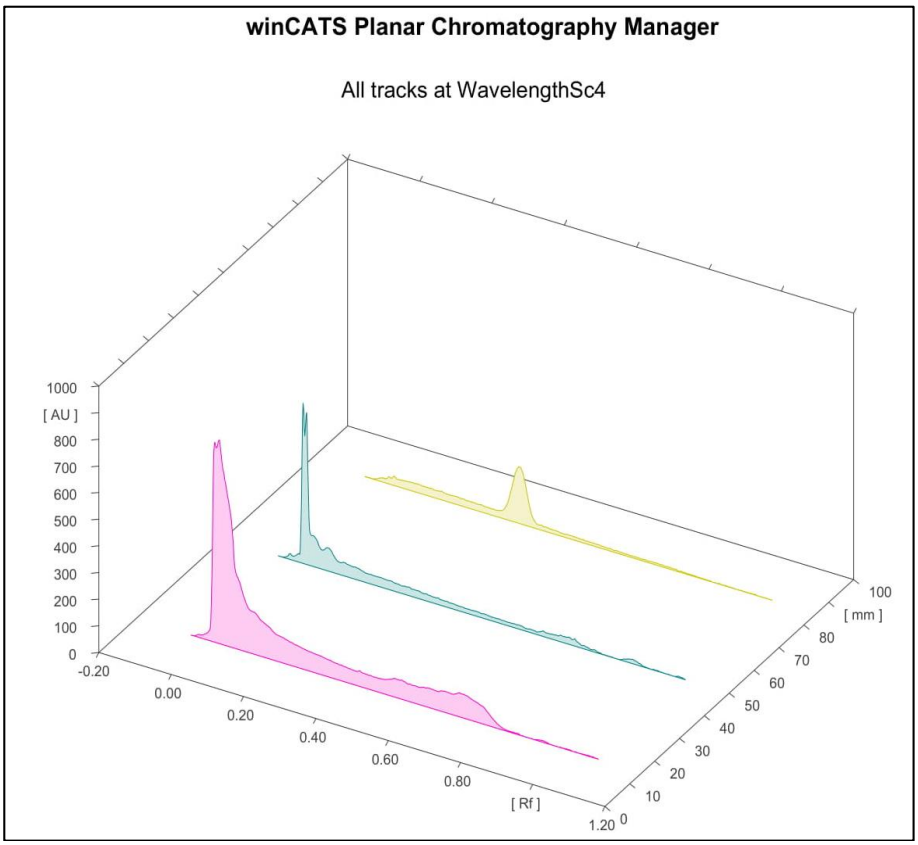


Fig 5: HPTLC fingerprint profile of all the tracks at 254nm of L. L. Leaf methanolic extract with mobile system chloroform-5ml, Ethyl acetate, -4ml, formic acid-1ml

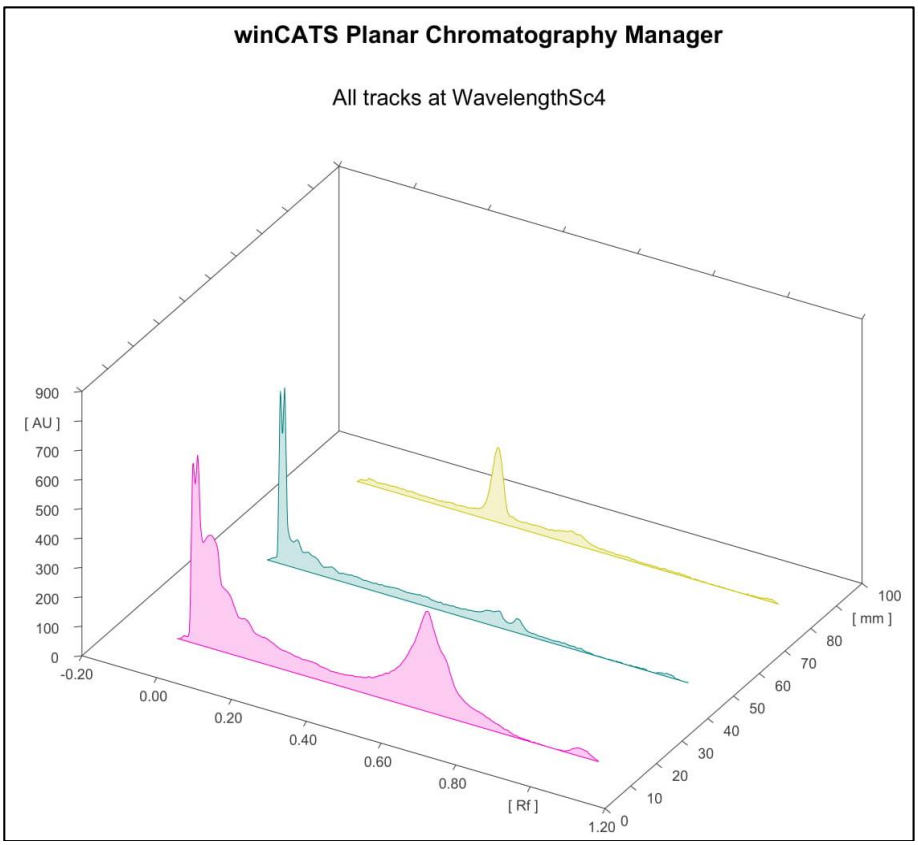


Fig 6: HPTLC fingerprint profile of all the tracks at 254nm of L. L. Leaf methanolic extract with mobile system chloroform-8.5ml, methanol-1ml, formic acid-0.5ml

Conclusion

The study was to detect the presence of useful compounds in *L. laevigata* which can be used as a drug by *in vitro* technique and. The phytochemical test showed a positive result for the primary metabolites such as proteins, carbohydrate, and amino acids are present in the appreciable amount in all the parts. Among the stem and leaf are used to seem therapeutically important part which possesses a higher

amount of secondary metabolites, such as phenolic compounds, flavonoids, tannins, saponins, steroid, and terpenoid.

The HPTLC fingerprinting of seeds of *L. laevigata* methanol extract was used as the sample, where the phenols, flavonoids showed the high number of peaks in maximum wavelength, *L. laevigata* leaf can be valuable natural high antioxidants properties source which seemed to provide potential therapeutical value for human health. Further, detailed exploration chemical studies and screening for medicinal and Anticancer against properties with providing a cost-effective and reliable source of medicine for the welfare of humanity.

Acknowledgements

The authors are grateful to Guide and Professor & Head, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu for providing necessary facilities during the study.

Authors Contributions

Sujatha designed the experiments performed in laboratory analysis, experiments, data analysis and participated in the writing of the manuscript. Dr. T. Sekar helped in paper writing and correction. Authors discussed the results and commented on the manuscript.

References

1. Amrit Pal Singh. Promising Phytochemicals from Indian Medicinal plants. Ethnobotanicals Leaflets, 2005, 1, Article 18.
2. Baquar SR. Medicinal and Poisonous plants of Pakistan, Pak. j. sci. ind. res. Ser. B: biol. sci. 2012; 55(3):129-137
3. Geisssman TA. Flavonoid compounds, Tannins, Lignins and related compounds, In M. Florkin and Stotz (Ed), Pyrrole Pigments, Isoprenoid compounds and phenolic plant constituents. Elsevier New York. 1963; 9:265.
4. Cook NC, Samman S. Flavonoids Chemistry, metabolism, cardio protective effects, and dietary sources. J. of Nutritional Biochemistry. 1996; 7(2):66-76.
5. Yadav R, Khare RK, Singhal A. Qualitative Phytochemical Screening of Some Selected Medicinal Plants of Shivpuri District (MP). Int J Life Sci Scienti Res. 2017; 3:844-847.
6. Farnsworth NR, Henry LK, Svoboda GH, Blomster RN, Yates MJ *et al*. Biological and phytochemical evaluation of plants. J Lloydia, 1966; 29:113-122
7. Singh SS, Pandey SC, Srivastava S, Gupta KS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi) Indian J Pharmacol, 2003; 35:83-91.
8. Lijun Wang, Curtis L Welle. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology. 2006; 17:300-312.
9. Wagner H, Bladt S. Plant drug analysis. A thin layer chromatography atlas. Springer, Berlin. 2001. 99-123
10. Vundac VB, Males Z, Plazibat M, Golja P, Cetina-Cizmek B. HPTLC determination of flavonoids and phenolic acids in some Croatian Stachys Taxa. J Planar Chromatogr. 2005; 18:269-273.
11. Nile SH, Park SW. HPTLC analysis, antioxidant and antigout activity of Indian plants. Iranian J Pharmaceutical Res. 2014; 12:531-539.
12. Shrivastava Manmohan. High performance thin layer chromatography (HPTLC). Springer publisher 2010, 3-4.
13. Sethis PD. HPTLC, first edition. CBS publishers, 1996. 4-5.
14. Thamaraiselvi P Lalitha, Jayanthi dP. Studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Asian Journal of Plant Science and Research. 2012; 2(2):115-122.
15. Singh Dharmendra, Poonam Singh Abhishek Gupta, Shikha Solanki, Ekta Sharma, Rajeev Nema. Qualitative Estimation of the Presence of Bioactive Compound in Centella Asiatica An Important Medicinal Plant. International Journal of Life Science and Medical Science, 2012; 2(1):5-7.
16. Judith Laure Ngondi, Emile Joachim Djiotsa, Zephyrin Fossouo, Julius Oben. Hypoglycaemic effect of the methanol extract of Irvingia gabonensis seeds on streptozotocin diabetic rats. African Journal of Traditional. 2006; 3(4):74-77.
17. Peter CH, Hollman. Evidence for health benefits of plant phenols: local or systemic effects. Journal of the Science of Food and Agriculture. 2001; 81(9):842-852.
18. Kokate CK, Gokhale AP, Purohit SB. A Textbook of Pharmacognosy, Nirali Prakashan: Pune, 2009, 174-180.
19. Ramann N. A Textbook of Phytochemical Techniques, NIPA: Pitam Pura, 2006, 19-24.