Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 867-874
Received: 08-09-2018
Accepted: 09-10-2018

Tarke Santosh Rangnathrao
Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, VELS Institute of Science, Technology and Advances Studies (VISTAS) Chennai, Tamil Nadu, India

Dr. P. Shanmugasundaram
Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, VELS Institute of Science, Technology and Advances Studies (VISTAS) Chennai, Tamil Nadu, India

Correspondence
Tarke Santosh Rangnathrao
Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, VELS Institute of Science, Technology and Advances Studies (VISTAS) Chennai, Tamil Nadu, India

Preliminary phytochemical screening and HPTLC method for qualitative determination of phytochemical compounds in extract of *Ehretia laevis* Roxb

Tarke Santosh Rangnathrao and Dr. P. Shanmugasundaram

Abstract
Background: The plant kingdom for its therapeutic use has been inadequately explored and there is a tremendous scope for the drug discovery for the human health problems. India has rich in plant wealth and also having excellent usage of medicinal plants with Ayurvedic science.
Objective: The objective of this study was to carry compound class detection present in plant extracts using chromatographic and by Phytochemical screening methods.
Materials and Methods: Preliminary phytochemical screening was done, physical constants were evaluated and HPTLC studies were carried out.
Results: Preliminary phytochemical screening and HPTLC analysis of the extract showed the presence of Triterpenes, Tannins, Saponins, Glycosides, Phenolic compounds and flavonoids.
Conclusions: It can be concluded that HPTLC analysis of extract can be used as an authentic diagnostic tool for the correct identification of secondary metabolites and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Keywords: Antioxidant, *Ehretia laevis*, HPTLC, phytochemical screening, physical constants

1. Introduction
Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters [1, 2]. 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 techniques and applying suitable standards [3, 4]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time *Ehretia laevis* Roxb is one of such plants which being used in Indian traditional medicine for the treatment of liver ailments. *Ehretia laevis*is an Indian medicinal plant [5, 8]. It is a deciduous shrub. It is considered as small tree due to its 12 m height belonging to the family Boraginaceous. Ehretia laevisis a small tree. It is generally found in Asia and Australian tropics. Literature survey revealed wide biological activity of family Boraginaceous. The inner bark of E. laevisis used as food. Leaves are applied to ulcers and in headache. Fruit is astringent, anthelmintic, diuretic, demulcent, expectorant and used in affections of urinary passages, diseases of lungs and spleen [9, 11]. Powdered kernel mixed with oil is a remedy in ringworm. Seeds are anthelmintic. This medicinal plant has an irregular trunk with a light grey or whitish bark. Leaves are variable in size and shape. They vary from 2 cm to 6.3 cm in length and 1.3 cm to 3.8 cm in width. Flowers of these plants are white in colour. The calyx of these flowers are 2.5 mm long, 3-lobed and the corolla are 6-8 mm long, in which 5 corolla are lobed. The tube and lobes of corolla are longer than the calyx [12, 14].
The present study was undertaken to Preparation of hydro alcoholic extract by Soxhlet extraction method and its sub fraction by successive extraction in n-Hexane, Ethyl acetate, and aqueous solvents. Phytochemical screening and quantitative estimation of phenolic and flavonoid content for hydro alcoholic extract, its fractions [15, 16]. Primary screening of phytochemical and qualitative analysis of secondary metabolites like Glycosides, Tannins, Saponins, Phenols, Triterpenoids, Flavonoids, Alkaloids and Steroids were carried out by HPTLC method [17].

2. Materials and methods

2.1 Plant Material

Flowers of *Ehretia laevis* plants, collected during the month of February 2018 from the Ambajogai, district Beed of Maharashtra state. Authenticated from Botanical Survey of India [Authentication number: No. BSI/WRC/100-2/Tech./2018/37], Ministry of Environment, Forest and Climate Change, Western regional center, Pune (Maharashtra) India.

2.2 Extraction procedure

The plant material of *Ehretia laevis* were dried at room temperature for fifteen days and then reduced to a coarse powder. This powder was used for the preparation of hydro alcoholic extract. The plant powder was extracted with 70% ethanol for 12 h at 50 ^{0}C. The obtained extract was concentrated under reduced pressure on rotary evaporator at 40^{0}C to obtain a brownish residue. The above extract was dissolved in distilled water and partitioned sequentially with n-hexane, ethyl acetate and aqueous to obtain n-hexane, ethyl acetate and aqueous fractions. All these fractions were concentrated using rotary evaporator. The yield of hydro alcoholic extract obtained by reflux method was found to be 23.25% w/w. The yield of n-hexane, ethyl acetate and aqueous fractions obtained by successive solvent-solvent extraction of hydro alcoholic extract was found to be 0.75, 9.5 and 15.35% w/w, respectively.

2.3 Quantification of total phenolic content

The total phenolic content of the extracts were determined with Folin- Ciocalteu (FC reagent).Reagents used are Folin-Ciocalteu (FC reagent), 20% Sodium carbonate solution and Gallic acid. Stock solution of Standard Gallic acid was prepared by 10mg of gallic acid was dissolved in 10ml of distilled water to obtain the concentration of 1mg/ml. Working solutions were prepared 50, 75, 100, 125, 150, 175, 200, 225, 250µg/ml. of the standard gallic acid were taken from the stock solution and the volume made up to 1ml with distilled water to obtain the final concentration of 50 250µg/ml. 1mg/ml of the test plant extracts were prepared. The volume is made up to 45 ml with distilled water in a volumetric flask. 1 ml of FC reagent (diluted 1:2 with distilled water) was then added and the content of the flask mixed properly. After three minutes, 3 ml of 20% sodium carbonate was added to the mixture and it was allowed to stand for 2 hour with occasional shaking. The absorbance of the blue colour that developed was read at 760nm in spectrophotometer.

2.4 Quantification of total flavonoid content

The total flavonoid content was determined by following the Aluminium chloride colorimetric methods described by Lobo *et al*, 2011. Reagents used are 5% Sodium nitrite solution, 10% Aluminium chloride solution and 1 M Sodium hydroxide. Stock solution of Standard Quercetin was prepared. Working solution of 10, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250µlof the standard Quercetin solution were taken from the stock solution and the volume made up to 1ml with distilled water to obtain the final concentration of 10-250µg/ml.1mg/ml concentration of the test plant extracts were prepared.2ml of distilled water was added in to the 1ml of the extracts and mixed properly. After 5 minutes 3ml of 5% sodium nitrite and 0.3ml of 10% aluminium chloride were added and stand for 6 minutes. After 6 minutes, 2ml of 1M sodium hydroxide was added to the solution and the volume was made up to 10 ml with distilled water. The red colored complex formed was measured at 510nm in spectrophotometer.

2.5 Phytochemical Screening

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative phytochemical analyses were done using the procedures of (Harborne, 1998) and (Khandelwal, 2005).The following tests were performed on extracts to detect various phyto - constituents present in them.

2.5.1 Detection of Flavonoid

2.5.1.1 Alkaline reagent test

To one ml solution of the extract 1 N NaOH solution was added to give yellow colour. This Colour vanishes after addition of few drops of dil. acid indicating the presence of Flavonoid.

2.5.2 Detection of Alkaloids

Solvent free extract, 50 mg was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows

2.5.2.1 Dragendorff's test

To a few ml of filtrate, 1 – 2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

2.5.3 Detection of Carbohydrates

The extract (100 mg) was dissolved in 5 ml of water and filtered. The filtrate was subjected to
The following tests.

2.5.3.1 Benedict's test to 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic colored precipitate indicated the presence of sugar.

2.5.4 Detection of Glycosides

For detection of glycosides, 50 mg of extract was hydrolysed with concentrated hydrochloric
Acid for 2 hrs. On water both, filtered and the hydrolysate was subjected to the following tests.

2.5.4.1 Keller Killiani test

The test solution with few drops of glacial acetic acid in 2 ml of 5% FeCl3 and concentration
H2SO4 from side of the test tube- Lower layer reddish brown and upper layer (bluish green)
Indicates the presence of Glycosides.

2.5.5 Detection of Saponins by Foam Test

The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of Saponins.

2.5.6 Detection of Proteins and Amino Acids

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through what man
No.1 filter paper and the filtrate were subjected to tests for proteins and amino acids.

2.5.6.1 Biuret test

An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this,1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, pink colour in the ethanolic layer indicated the presence of proteins.

2.5.7 Detection of phenolic compounds and Tannins

2.5.7.1 Ferric chloride test

The extract (50 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% Ferric chloride solution was added. A dark green colour indicated the presence of phenolic Compounds.

2.5.8 Detection of Phytosterols

2.5.8.1 Salkowaski test

To 2ml of test extract, 2 ml of chloroform and few drops of conc. H_2SO_4 were added, Shaken and allowed to stand lower layer turns red indicating the presence of sterols and yellow colour indicates the presence of terpenoids.

2.6 HPTLC Screening

Table 1: Plate layout of Phytoconstituents

Parameters →	Stationary phase	Plate format	Application	Track
Alkaloids	Merck, TLC Plates silica gel 60 F 254	100.0*100.0mm	position y:8.0mm, distance:8.0mm, width:0.0	First position x:20.0mm, distance:19.4mm First position x:20.0mm, distance:19.4mm
Flavonoids	Merck, TLC plates silica gel 60 f 254	100.0*100.0mm	Position Y:8.0mm, width:0.0mm	First position*20.0mm, distance 19.mm
Phenols	Merck, TLC Plates silica gel 60 F 254	100.0*100.0mm	Position Y: 8.0mm, length:8.0mm, width:0.0mm	First position X:20.0mm, distance:19.4mm
Glycosides	Merck, TLC Plates silica gel 60 F 254	100.0*100.0mm	Position Y:8.0mm, length:8.0mm, width: 0.0mm	First position X:20.0mm, distance:19.4mm
Saponins	Merck, TLC plates silica gel 60 f 254	100.0*100.0mm	Position Y:8.0mm, length:8.0mm, width: 0.0mm	First position X:20.0mm, distance:19.4mm
Steroids	Merck, TLC Plates silica gel 60 F 254	100.0*100.0mm	Position Y:8.0mm, length:8.0mm, width: 0.0mm	First position X:20.0mm, distance:19.4mm
Tannins	Merck, TLC Plates silica gel 60 F 254	100.0*100.0mm	Position Y:8.0mm, length:8.0mm, width: 0.0mm	First position X:20.0mm, distance:19.4mm
Triterpenoids	Merck, TLC Plates silica gel 60 F 254	100.0*100.0mm	Position Y:8.0mm, length:8.0mm, width: 0.0mm	First position X:20.0mm, distance:19.4mm

Solvent front position 70mm.

Table 2: Development 1 chamber for Phytoconstituents

Parameters →	Mobile Phase	Saturation time	Volume front through	Volume rear through	Drying time
Alkaloids	Toluene: Ethyl Acetate: Diethylamine (7:2:1 v/v/v)	20 min	10ml	20ml	5min
Flavonoids	Ethyl acetate: formicacid: glacial acetic acid: water (10:0.5:0.5:1 v/v/v/v)	20 min	10ml	20ml	5min
Phenols	Cyclohexane: ethylacetate: formic acid (4:6:1 v/v/v)	20 min	10ml	20ml	5min
Glycosides	Ethyl acetate: methanol: water (10:1:4:1 v/v/v)	20 min	10ml	20ml	5min
Saponins	Chloroform: Acetic acid: Methanol: Water (6.8:3.2:1.4:0.8 v/v/v/v	20 min	10ml	20ml	5min
Steroids	n- Butanol: Methanol: Water (3:1:1 v/v/v)	20 min	10ml	20ml	5min
Tannins	Toluene: ethyl acetate: formic acid (6:4:0.3 v/v/v)	20 min	10ml	20ml	5min
Triterpenoids	n – Hexane: Ethyl Acetate (1:1 v/v)	20 min	10ml	20ml	5min

Drying temperature: Room temperature

Table 3: Derivatization 1 for Phytoconstituents

Parameters	Reagent name	Dipping speed	Dipping time	Heating
Alkaloids	Dragendorff's reagent	2.0ml	0 s	None
Flavonoids	NP Reagent	2.0ml	0 s	None
Phenols	ASR	5.0ml	0 s	100 degree c for 30 min, heat after
Glycosides	Alcoholic KOH	5.0ml	0 s	None
Saponins	ASR	5.0ml	0 s	100 ^{0}C for 3 min, heated after
Steroids	ASR	5.0ml	0 s	100 ^{0}C for 3 min, heated after
Tannins	Alcoholic Fecl3	5.0ml	0 s	None
Triterpenoids	ASR	5.0ml	0 s	None

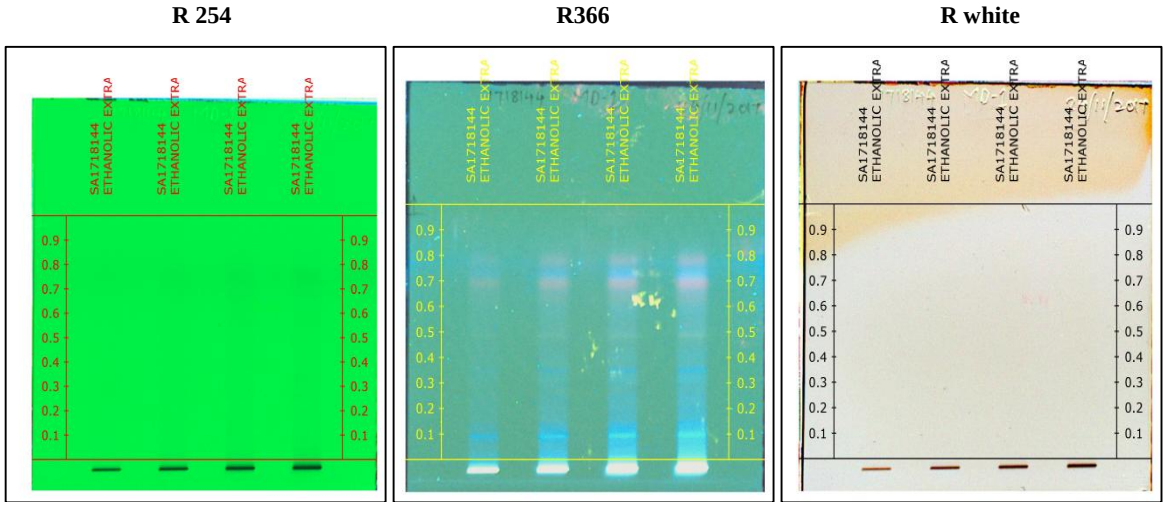

Fig 1: HPTLC profile of Alkaloids

Peak	start		Max			End		Area		Manual peak
#	Rf	H	Rf	H	%	Rf	H	A	%	
1	0.002	0.0000	0.015	0.0519	56.26	0.043	0.0180	0.00115	38.43	no
2	0.667	0.0009	0.736	0.0404	43.74	0.783	0.0034	0.00184	61.57	no

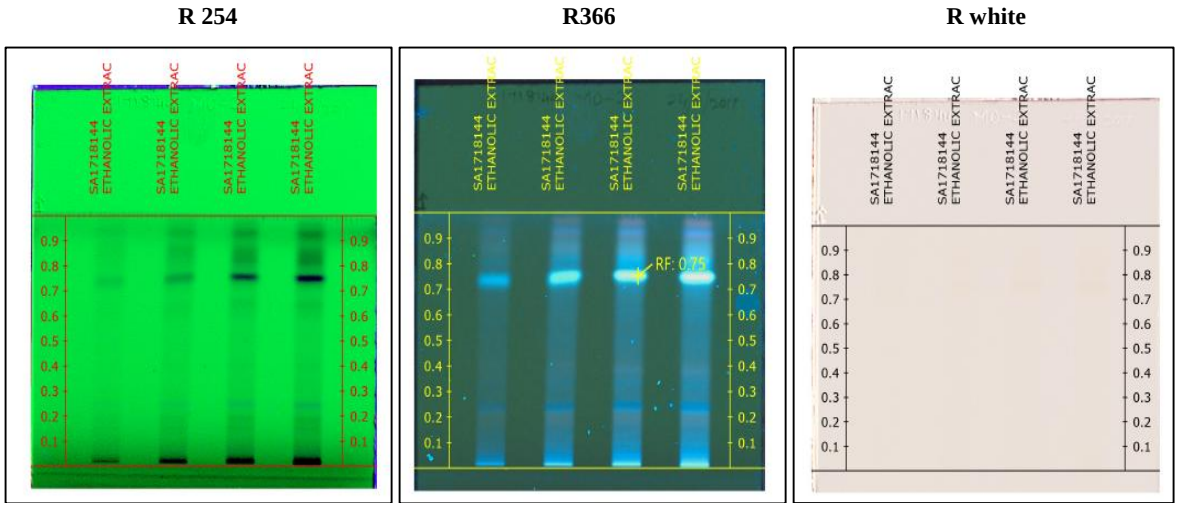

Fig 2: HPTLC profile of Flavonoids

Peak	Start		Max			End		Area		Manual peak
#	Rf	H	Rf	H	%	Rf	H	Rf	H	
1	0.002	0.0000	0.012	0.0914	45.93	0.111	0.0094	0.00312	40.46	no
2	0.398	0.0012	0.406	0.0141	7.07	0.417	0.0007	0.00013	1.68	no
3	0.680	0.0026	0.746	0.0935	47.00	0.792	0.0101	0.00446	57.86	no

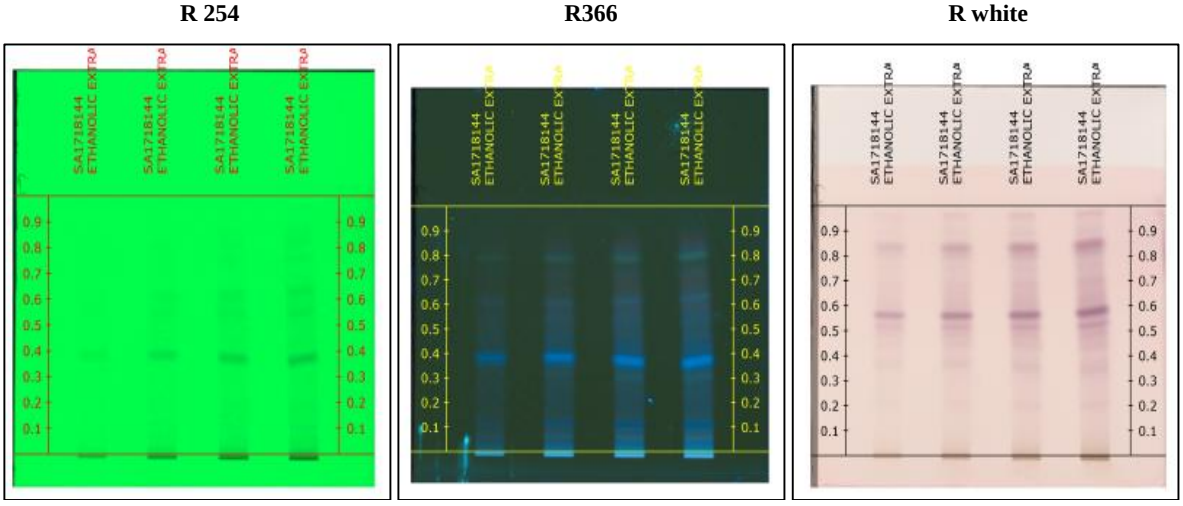

Fig 3: HPTLC profile of Phenols

Peak	Start		Max			End		Area		Manual peak
#	Rf	H	Rf	H	%	Rf	H	A	%	
1	0.002	0.0000	0.012	0.1142	31.77	0.043	0.0354	0.00252	23.72	no
2	0.714	0.0148	0.755	0.2453	68.23	0.787	0.0282	0.00810	76.28	no

R 254 **R366** **R white**

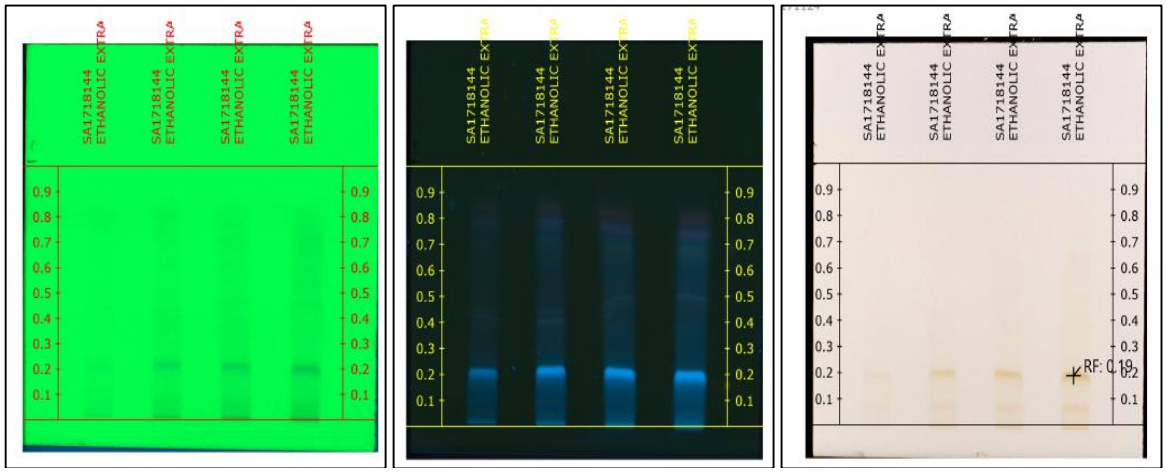

Fig 4: HPTLC profile of Glycosides

Peak	Start		max			End		Area		Manual peak
#	Rf	H	Rf	H	%	Rf	H	A	%	
1	0.002	0.0000	0.010	0.1040	21.16	0.038	0.0167	0.00183	11.90	no
2	0.682	0.0117	0.749	0.3873	78.84	0.783	0.0493	0.01355	88.10	no

R 254 **R366** **R white**

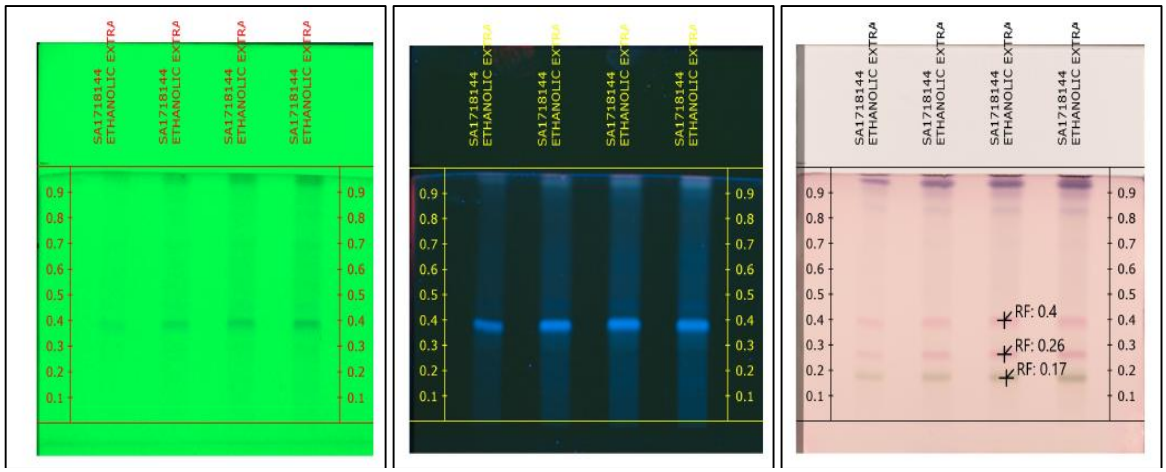

Fig 5: HPTLC profile of Saponins

R 254 **R366** **R white**

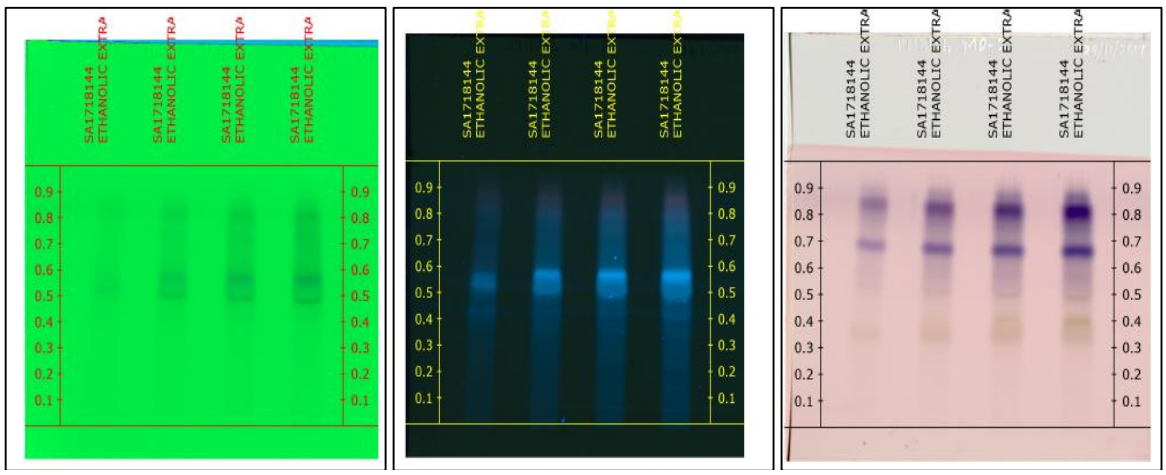

Fig 6: HPTLC profile of Steroids

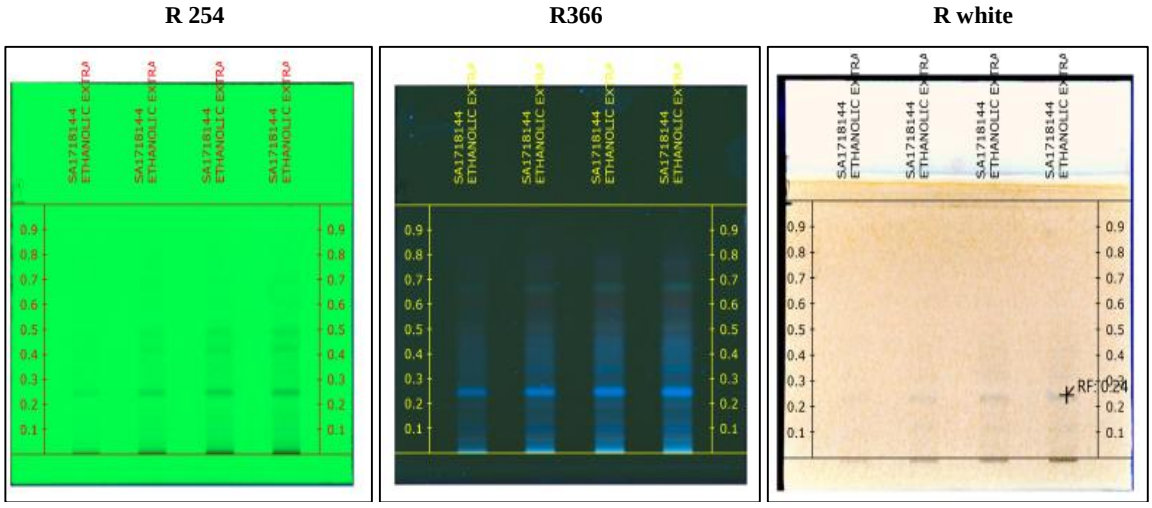

Fig 7: HPTLC profile of Tannins

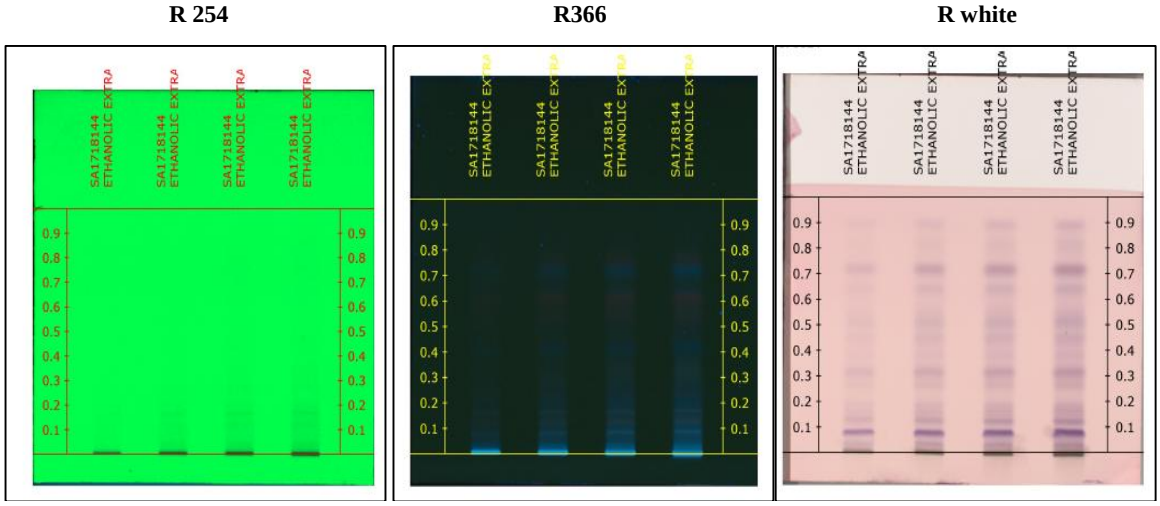

Fig 8: HPTLC profile of Triterpenoids

3. Result and Discussions

3.1Preparation of Hydroalcoholic extract and fractions

The yield of Hydroalcoholic extract obtained by reflux method was found to be 21% w/w. Theyield of n-hexane, chloroform, n-butanol and aqueous fractions obtained by successive solvent extraction of Hydroalcoholic extract was found to be 5.5, 5.5, 4.5 and 84.4% w/w, respectively.

Table 5: Solvent extraction of Hydroalcoholic extract

Name of extract	Description	polarity	weight
Hydroalcoholic extract	Brown	70% ethanol	23.25 gm
N-hexane fraction	Green	100% Nhexane	0.2 gm
Ethyl acetate	army	100% Ethyl acetate	2.21 gm
Aqueous fraction	Florescent brown	100% water	3.57 gm

3.2 Quantification of total phenolic content

The concentration of total phenols was expressed as Gallic acid equivalents in mg/gm of dry extracts.

3.2.1 UV visible spectroscopy standard curve

Sr. No	Sample	Abs. 780nm		µg/ml
1	Blank	0.028	-	-
2	Aqueous	0.449	0.421	43.8
3	Ethanolic	0.653	0.625	64.2
4	Ethyl acetate	0.240	0.232	22.3
5	n-Hexane	0.104	0.076	9.3

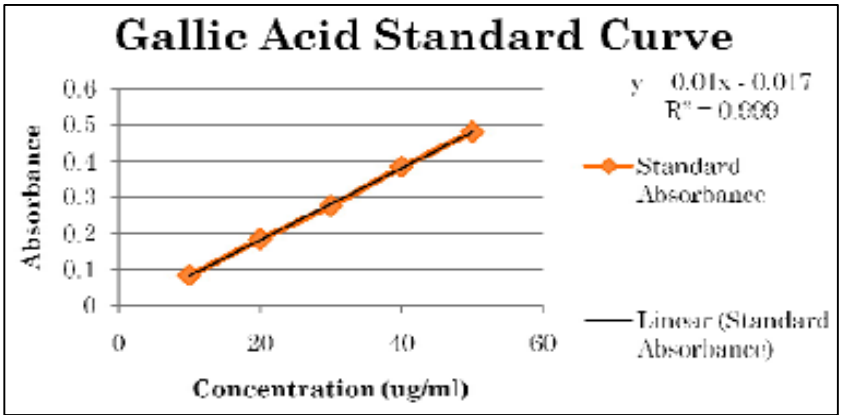

Fig 9: Standard calibration curve of Gallic acid

3.3 Quantification of total flavonoid content

Thepercentage of totalflavonoids were calculated from the standard calibration curve of Quercetin.

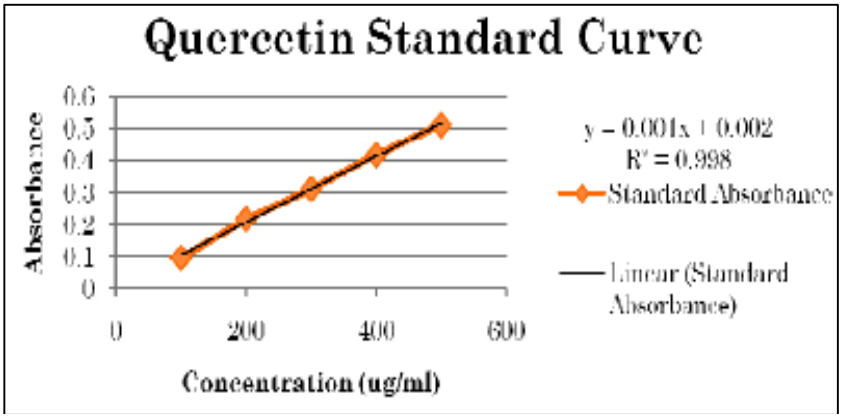

Fig 10: Standard calibration curve of Quercetin

Sr. No	Sample	Abs. 780nm		µg/ml
1	Blank	0.008	-	-
2	Aqueous	0.301	0.293	291
3	Ethanolic	0.903	0.895	853
4	Ethyl acetate	Turbid	--	--
5	n-Hexane	Turbid	--	--

3.4 Phytochemical Screening

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The tests show following results.

Table 6: Phytochemical Screening

Sr. No.	Test name	Test for	Aqueous Extract	Ethanolic Extract	Ethyl acetate Extract	n-Hexane Extract
1	Alkaline reagent test	Flavonoids	+	+	+	-
2	Dragendoff's test	Alkaloids	-	+	-	-
3	Fehling's test	Carbohydrates	++	+	-	-
4	Benedict's test	Reducing Sugar	+	-	-	-
5	Keller Killiani test	Glycosides	-	++	+	-
6	Foam test	Saponins	++	+	-	-
7	Biuret test	Proteins	-	-	-	-
8	$FeCl_3$ test	Phenolic compounds	+	+	-	-
9	Salkowaski test	Triterpenoids	-	-	+	-
		Sterols	-	+	+	+

3.5 HPTLC Screening

3.5.1 Alkaloids

After Derivatization with Dragendorff's reagent, brown or orange-ish brown colour bands are observed in Alkaloids. Here none of the above colors were observed, hence it was concluded that Alkaloids are absent in the given sample.

3.5.2 Flavonoids

After derivatization with Natural product reagent fluorescent bands are observed in Flavonoid compounds. As Fluorescent bands were observed it was concluded that Flavonoids are present in the given sample

3.5.3 Phenols

Phenols give Violtish Blue band after derivatising in ASR. Hence we can see some bands with positive results in the sample.

3.5.4 Glycoside

Glycosides show positive colour as bottle green bands after derivatising in Alcoholic KOH we can see a band with positive colour in the sample.

3.5.5 Saponins
Saponins give pinkish, greenish band after derivatising in ASR. We can see positive result in the plate.

3.5.6 Steroids
Violet bands were observed in the given sample after derivatization with Anisaldehyde Sulphuric acid Reagent (ASR). Hence it can be concluded that Steroids are present in the given sample.

3.5.7 Tannins
Tannins shows Black colored band after derivatising in Alcoholic Fecl3. Thus at rf 0.24 we can see a positive result.

3.5.8 Triterpenoids
Triterpenoids shows Bluish and Violet band after derivatising in ASR we can see plenty bands with positive result in the plate.

4. Discussion
Authentication of medicinal plants at chemical and genetic level is a crucial step for both research purposes and commercial preparations. Recently anatomical, biochemical, cytological, and molecular markers are also being used to classify the organisms in addition to morphological markers. Mostly, the entire chromatographic profiles are used to identify or to evaluate the quality of the herbs investigated. A thorough understanding of their chemical composition is essential for conducting a safety risk assessment. The chemical constituents and their amounts in herb can be different, due to growing conditions, such as climate, soil fertility, the harvest season, age of the leaves, the drying process, etc. HPTLC studies have shown that it is more resourceful than ordinary TLC methods, as the spots are well resolved. It is an invaluable quality assessment tool for the assessment of botanical materials, and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. It is helpful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The exclusive characteristic of the picture like image of HPTLC coupled with digital scanning profile is progressively attractive to herbal analysis to construct the herbal chromatographic fingerprint. To our knowledge HPTLC profile of *Ehretia Laevis* generated in ethanol solvent system in order to establish the total number of bioactive compounds. The chromatographic finger prints obtained can be stored as electronic images without any errors and change for further usage.

5. Conclusion
To conclude identification of secondary metabolites can be done with Phytochemical screening and authenticated by HPTLC method. For the selected plant *Ehretia laevis* have shown presence of flavonoids, phenolic compounds, Saponins, triterpenoids and steroids which are confirmed by HPTLC analysis and these constituents have antioxidant activity and other medicinal properties hence the study can further be taken up to explore this medicinal plant in depth.

6. References

1. Philipson JD, Charlwood BV, Rhodes MJ. Plants as sources of Valuable products, Secondary Products from Plant tissue Culture, Oxford, Clarendon Press, 1990, 1-22.
2. Admuthe NB, Nalwade AR. Micropropagation for *Ehretia laevis* Roxb. A rare Indian medicinal plant. International Journal of Advanced Scientific and Technical Research. 2016; 6(3):411-422.
3. Ulyana A, Daniel E, Michel H, Edward J, Kennelly S. Antioxidant activity of browning reaction prepared from glucosamine, Phytotherapy Research. 2002; 16(3):63-65.
4. Kamble GS, Upadhye SV, Deshpande NR. *In-Vitro* Antioxidative Activity of Phenolic and Flavonoid Contents of Leaves of Medicinal Plant – *Ehretia laevis.* International Journal of Pharma and Bio Sciences. 2011; 2(1):810-815.
5. Jyothirmai N, Nagaraju B, Yasodeepika M, Suresh Kumar J, Rani SG. Evaluation of Anti-Inflammatory and Anti-Bacterial Activities of Different Solvent Extracts of *Ehretia laevis* Roxb. Journal of Pharmaceutical Sciences and Research. 2016; 8(8):715-720.
6. Torane RC, Kamble GS, Khatiwora E, Ghayal NA, Deshpande NR. Antioxidant Capacity of Leaves and Stem of *Ehretia laevis.* International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(2):233-236.
7. Ahmad VU, Rashid MA, Abbasi MA, Rasool N, Zubair M. New salirepin derivatives from *Symplocos racemosa*. Journal of Asian Natural Products Research. 2007; 9(3):209-215.
8. Pimpliskar MR, Jadhav RN, Jadhav BL. Study on antimicrobial principles of Rhizophora Species along Mumbai Coast. Journal of Aquatic Bio technology. 2011; 26(1):6-11.
9. Sharma B, Gupta S, Sharma K. Natural folk remedy for menorrhagia. Journal of information, knowledge and research in humanities and social service. 2011; 2(1):86-88.
10. Shetty P, Palve A, Jadhav RN, Shinde P, Pimpliskar M. In-Silico docking analysis of *S. lychnophora* compounds against protein causing Alzheimer's disease. International Journal of Engineering Science and Innovative technology. 2014; 3(4):158-164.
11. Bhutani KK, Jadhav AN, Kalia V. Effect of *Symplocos racemose* Roxb. On gonadotropin release in immature female rats and ovarian histology. Journal of Ethno pharmacology. 2004; 94(1):197-200.
12. Ahmad UV, Abbasi MA, Hussain H, Akhtar MN, Farooq U, Fatima N *et al*. Phenolic glycosides from *Symplocos racemosa*: natural inhibitors of phosphodiesterase –I. Phytochemistry. 2003; 63(2):217-220.
13. Sathiavelu M, Arunachalam S. High performance thin layer chromatography profile of *Cassytha filiformis*. Asian Pacific Journal of Tropical Biomedicine, 2012, S1431-S1435.
14. Goodarzi M, Russel PJ, Heyden YV. Similarity analyses of chromatographic herbal fingerprints: A review. Analytica Chimica Acta. 2013; 804:16-28.
15. Khandelwal KR. Practical Pharmacognosy. 12th ed. Pune: Nirali prakashan, 2004, 149-160.
16. Dinesh Kumar C. Pharmacognosy can help minimize accidental misuse of herbal medicine. Current Science. 2007; 93:1356-1358.
17. Elangovan M, Dhanarajan MS, Rajalakshmi A, Jayachitra A, Pardhasaradhi M, Narasimharao B. Analysis of Phytochemicals, Antibacterial and Antioxidant activities of *Moringa oleifera* Lam. Leaf extract- an *in vitro* stud. International Journal of Drug Development and Research. 2014; 6(4):280-285.