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Phytochemical Screening and HPTLC Fingerprinting Profile of bark extracts of *Ehretia laevis*

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Abstract

To establish the Preliminary Phytochemical Screening and fingerprint profile of bark Extracts of *Ehretia laevis by* using high performance thin layer chromatography (HPTLC) technique. Preliminary phytochemical screening of extracts were done, physical constants were evaluated and HPTLC studies were carried out. CAMAG make HPTLC system equipped with Linomat 5 applicator, TLC scanner 3, server vision CATS-server PH, version 2.5.18072.1 software were used. Preliminary phytochemical screening of the extract showed the presence of Flavonoids, glycosides, tannins, alkaloids, terpenoids, phenolic compounds, proteins, reducing sugars, fats and oils. The fingerprint analysis of petroleum ether extract showed four peaks, chloroform extract showed four peaks and methanolic extract showed six peaks in 10 µl of the sample analysed. It can be concluded that HPTLC fingerprint analysis of Petroleum ether, chloroform and methanolic extract of bark powder of *Ehretia laevis* 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.

Key- words: Phytochemical screening, Physical constants, HPTLC, Petroleum ether extract, chloroform extract, methanolic extract, *Ehretia laevis*

Introduction

India has a cultural rich heritage of traditional medicine comprised of two widely flourishing systems of Ayuvedic and Unani systems.[1] As per WHO guideline, it has been emphasized that there is need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards. [2] Standardization of plant drug materials is the need of the day. Several pharmacopoeias which contains monographs of the plant materials they describe only the physicochemical parameters, hence the modern methods which are describing the identification, quantification structutal determination of active constituents in the plant material may be useful for proper standardization of herb and its formulations. [3, 4] HPTLC fingerprinting technique offers better resolution and estimation of important active constituents which can be done with reasonable accuracy in a shorter time . [5] Plants used in traditional medicine have stood up to test of time and contributed many novel phytochemical compounds for preventive and curative medicine to modern science. India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. [6]

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Medicinal plants have been in use from time immemorial and their utilisation has been increasing day by day in the present time. Naturally obtained compounds are use as safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs is also reduced. Plants always represent a source of lead compounds for many pharmaceuticals, phytochemical compounds and secondary metabolites present in plants have been used in treating a number of human ailments. [7]

Herbal medicines widely used in health-care in both developed and developing countries are complex chemical substances prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally. According to the estimation of World Health Organization (WHO), about 80% of the world population uses herbs and other traditional medicines for their primary health care needs. Herbal drugs are finished labeled products that contain chemically active constituents such as aerial, sub-aerial and underground parts of plant or other plant material or combination thereof, whether in the crude state or as herbal preparations. [8]

Phytochemical substances are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds

found in plants. Preliminary phytochemical screening assay is a simple, quick and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemical substances in a mixture and an important tool in bioactive compound analysis. [9]

HPTLC technique is a more efficient, faster with more reliable and reproducible results. HPTLC with latest digital scanning profile, it also also provides accurate and precise retention factor (R_f) values and quantitative determination of sample by *in situ* scanning densitometry aided by formation of easily detected derivatives by post-chromatographic chemical reactions as needed, as well as a record of the separation in the form of a chromatogram with fractions represented as peaks with defined parameters including absorbance, R_f value, height and area. [10] HPTLC fingerprinting analysis could be used in proper identification of medicinal plants, as a valuable analytical tool in the routine quality control and standardization of herbal drugs[11] and as a chemotaxonomical tool in the plant systematic[12], for determination of medicinally bioactive components of the herbal medicine. [13] The present study was aimed to investigate HPTLC Fingerprinting profile of Petroleum ether, Chloroform and Methanolic extract of bark of plant *Ehretia laevis* for analyzing marker chemical constituents.

Ehretia laevis is a small tree belonging to family Ehretiaceae. The plant is native to India, Pakistan, Myanmar, Vietnam, China, Bhutan. The plant *Ehretia laevis* is highly located at hilly forests, in ravine and on hill slopes. The plant is known as Dant-Rang, Vadhvarni, Chamror. [14] The inner bark of plant *E. laevis* is used as food and nutritive. Leaves of plant is applied to ulcers, skin diseases and in headache. Fruit of plant is used as urinary passage problem, lung and spleen diseases, astringent, anthelmintic, diuretic, demulcent, expectorant. Powder of kernel of plant is mixed with oil is a remedy in ringworm. Seeds are used as treatment for earthworms. Barks of plant is used in infection of throat. Root is used for treatment for veneral diseases. The plant contains chemical constituents like as alkaloids, fatty acids, phenolic acids, flavonoids, cyanogenetic glycosides, and benzoquinones. [15, 16]

Material and Methods

Plant Collection

The fresh barks of plant *Ehretia laevis* were collected from haripura and manudevi region of Taluka Yawal, District Jalgaon, India. The selected plants were authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule, Maharashtra . Barks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Preparation of Plant extract

The extraction process was carried out using continuous soxhlet extraction method. About 300 gm of dry powdered plant material or bark was extracted in Soxhlet apparatus with 500ml of petroleum ether for 16 hours and successively with chloroform and methanol as solvent. After extraction, the solvent was removed using rotary vacuum evaporator to give a concentrated extract at 60˚C in a water bath. It was then dried aseptically with the help of drier and subjected to Preliminary phytochemical screening and HPTLC fingerprinting. [17]

Phytochemical Screening

The different qualitative phytochemical tests were used for identification of the phytoconstituents present in the petroleum ether, chloroform and methanolic extracts of *Ehretia laevis* barks for the identification of the various active chemical constituents like as alkaloids, phenols, flavonoids, tannins, terpenoids, glycosides, reducing sugars, fats and oils. [18] The positive tests were noted as present (+) and absent (-).

Evaluation of physical constants[18]

Foreign Matter, Moisture Content, Total Ash Value, Water Soluble Ash Value, Acid Insoluble Ash Value, Alcohol soluble Extractive Value Water soluble extractive value were carried out. [19] The results are presented in Table 2.

HPTLC Fingerprinting Equipments [20, 21]

Sample preparation: The plant extracts residue was redissolved in 1ml of chromatographic grade methanol, which was used for sample application on Merck HPTLC plates pre-coated silica gel 60F 254 aluminium sheets.

Developing solvent system: A number of solvent systems were tried for petroleum ether, chloroform and methanolic extract, but the satisfactory resolution was obtained in the solvent system Toluene: Methanol (9:1) for petroleum ether extract, Toluene : Ethyl alcohol (7:3) for chloroform extract and Toluene:Ethyl acetate:Methanol:formic acid (5:4:1:1) for methanolic extract,.

Sample application: Application of bands of each extract was carried out using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F 254 aluminium sheets (20 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG make HPTLC system, which was programmed

through Vision CATS software (2.5.18072.1). The samples (10µl) were spotted in the form of bands of width 8mm with a 100 microlitre sample using a Hamilton syringe.

Development of chromatogram: After the application of sample, the chromatogram was developed in Twin trough glass chamber 20x 10 cm saturated with the solvent system Toluene: Methanol (9:1) for petroleum ether extract, Toluene : Ethyl alcohol (7:3) for chloroform extract and Toluene:Ethyl acetate:Methanol:formic acid (5:4:1:1) for methanolic extract for 20 min.

Detection of spots: The air-dried plates were viewed in ultraviolet radiation to mid day light. The chromatograms were scanned by densitometer at 366nm and R White in Densitometry TLC Scanner4. The R_f values and finger print data were recorded by Vision CATS software.

Results and Discussion

The preliminary qualitative phytochemical screening of the crude powder of *Ehretia laevis* was done to assess the presence of bioactive components. The presence of various phytoconstituents like alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds was determined (Table 1).

Table 1: Preliminary phytochemical screening on petroleum ether, chloroform and methanolic bark extracts of *Ehretia laevis*

Sr. No	Phytochemical constituents	Petroleum ether extract	chloroform extract	methanolic extract
1	**Alkaloids**			
	a) Dragendorff's test	+	++	+++
	b) Mayer's test	+	++	+++
	c) Hagers's test	-	-	-
	d) Wagner's test	+	+	++
2	**Carbohydrates**			
	a) Molisch's test	-	+	+
	b) Fehlings test	-	+	+
	c) Benedict test	-	+	+
3	**Saponins**			
	a) Foam Test	-	-	+
	b) Heamolytic test	-	-	+
4	**Steroids and Tri terpenoids**			
	a) Salkowski test	++	++	-
	b) Liebermann – Burchard Reaction	++	++	-
	c) Lieberman's Reaction	++	++	-
5	**Phenolic compounds & Tannins**			
	a) Ferric chloride test	-	++	+++
	b)Lead acetate test	-	++	+++
	c) Potassium Dichromate test	-	+	+++
	d) Dilute HNO3	-	+	+++
6	**Proteins & Amino acids**			
	a) Biuret test	-	+	+
	b) Millions test	-	+	+
	c) Ninhydrin test	-	+	+
7	**Flavone & Flavanoids**			
	a) Lead acetate test	-	+	+++
	b) Ferric chloride test	-	+	+++
	c) Sodium Hydroxide test	-	+	++
	d) Shinoda test	-	+	++
8	**Anthraquinone Glycosides**			
	Borntrager's test	-	-	-

+++ = maximum; ++ = moderate; + = minimum; - = absent

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Foreign Matter, Moisture Content, Total Ash Value, Acid Insoluble Ash Value, Water Soluble Ash Value, Soluble, Alcohol soluble Extractive Value, Water soluble extractive value were carried out**.** The results are presented in Table 2.

Table 2: Physical constants for bark of plant *Ehretia laevis*

Sr. No.	Evaluation Parameter	Value (%)
1	Foreign matter	0.2
2	Moisture content	6.3
3	Total Ash value	10.45
4	Acid insoluble Ash Value	1.13
5	Water Soluble Ash Value	6.42
6	Alcohol soluble extractive value	11.55
7	Water soluble extractive value	7.17

The HPTLC chromatogram, peaks, R_f values and area obtained for solvent extracts after scanning are depicted in respective figures (1 and 2) and Table (3, 4 and 5). The chromatograms of *Ehretia laevis* revealed that all sample constituents were clearly separated without any tailing and diffuseness. It is evident from Table 2 that in petroleum ether extract of *Ehretia laevis* barks, there are four peaks indicating the occurrence of at least four different components in petroleum ether extract. The components with R_f values 0.082, 0.193, 0.358 and 0.963 were found to have % area ranging between 14.68 and 21.58 as shown in Fig. 1. The chloroform extract exhibited four spots indicating the occurrence of at least four different components. In this case, the components with R_f values 0.526, 0.640, 0.900 and 0.963 were found to be more predominant as the % area was ranging from 9.64 to 40.60. The methanolic extract exemplified six peaks at the following R_f values 0.013, 0.043, 0.091, 0.130, 0.606 and 0.637 indicating the occurrence of six different components in this extract as the % area was ranging from 6.5 to 54.91.

HPTLC finger printing is an valuable quality assessment tool for the evaluation of botanical materials, 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 are well resolved. The HPTLC method is simple, rapid, accurate, reproducible, selective and economic, can be used for quality control analysis[19] and for quantitative determination of the plant material.

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.

Table 3: R_f values of peak formed of *Ehretia laevis* Petroleum ether extract

Sr. No.	Peak	R_f	Height	Area
1	1	0.082	0.0420	19.37
2	2	0.195	0.0962	44.37
3	3	0.358	0.0318	14.68
4	4	0.963	0.0468	21.58

Table 4: R_f values of peak formed of *Ehretia laevis* Chloroform extract

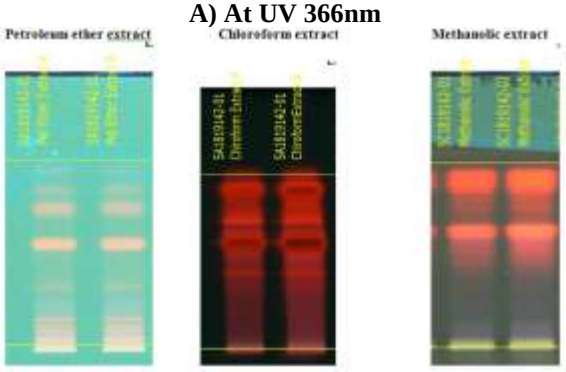
Sr. No.	Peak	R_f	Height	Area
1	1	0.526	0.2741	31.20
2	2	0.640	0.3567	40.60
3	3	0.900	0.1631	18.56
4	4	0.963	0.0847	9.64

Table 5: R_f values of peak formed of *Ehretia laevis* Methanolic extract

Sr. No.	Peak	R_f	Height	Area
1	1	0.013	0.2229	54.91
2	2	0.043	0.0320	7.89
3	3	0.091	0.0264	6.5
4	4	0.130	0.0207	5.09
5	5	0.606	0.0518	12.77
6	6	0.637	0.0520	12.82

Figure 1: HPTLC finger print profile of different solvent extracts of bark of *Ehretia laevis* at UV 366 nm and R White

A) At UV 366nm



B) At R White

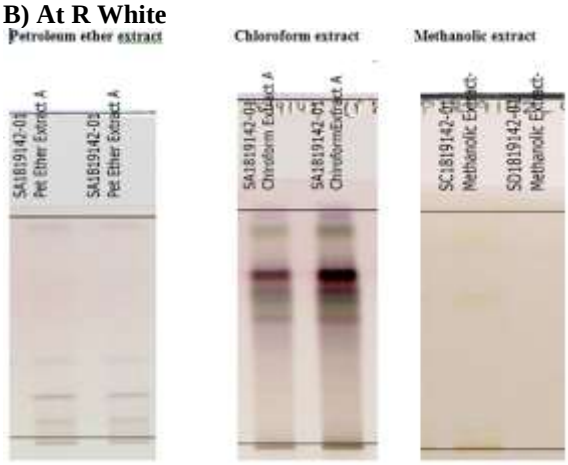
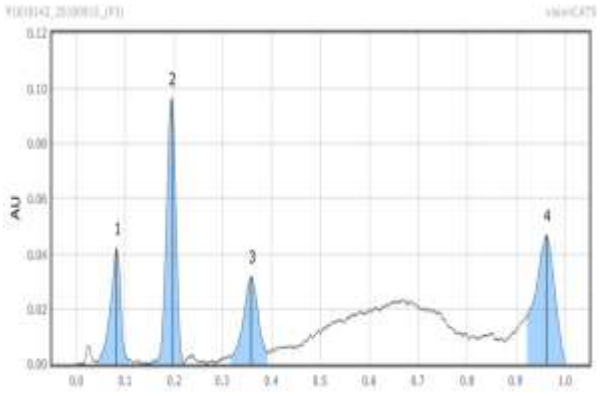
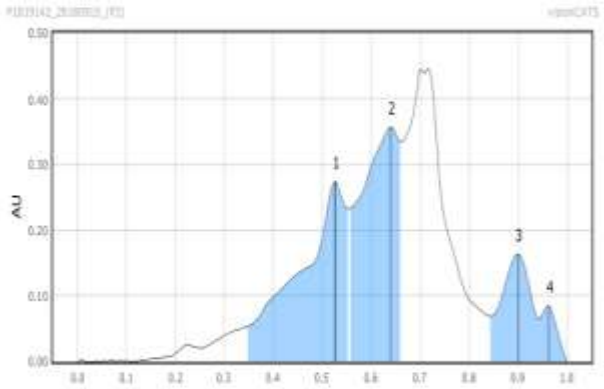


Figure 2: HPTLC chromatogram of different solvent extracts bark of *Ehretia laevis* at UV 366 nm.

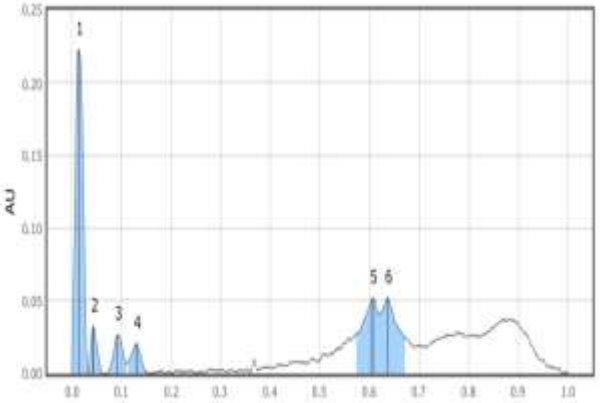
A) Petroleum ether extract



B) Chloroform extract



C) Methanolic extract



Conclusion

HPTLC fingerprint is a best technique to check the genetic variability present in various plant species. It is a simple, renewable, less cost, defined, exact method in identifying a various plant species and can also be used in standardization, characterization and authentication of medicinally important plants. HPTLC finger printing is helps in differentiating the adulterant, substitutes and species. It can serve as a biochemical marker for *E. laevis* in the plant studies and pharmaceutical companies. Further research is to carry out characterization of the phytoconstituents and to execute quantitative estimation with the help of marker compounds is also important, but the HPTLC fingerprinting data from the present study could be considered for setting up standards to *E. laevis.*

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