

HPTLC ANALYSIS OF ETHANOLIC EXTRACT OF LEAVES OF *CELASTRUS PANICULATUS***Amjad Pasha¹, Dr. Pavan Kumar², Dr. Sadath Ali³, Dr. Seema Firdouse⁴, Dr. Parwez Alam⁵ and Buthul Fathima⁶**¹Research Scholar, Department of Pharmaceutical Sciences, Singhania University, Pachheri Bari, Jhunjhunu, Rajasthan India.²Professor, Department of Pharmaceutical Sciences, Singhania University, Pachheri Bari, Jhunjhunu, Rajasthan India.³Professor, Department of Pharmaceutical Chemistry, MAM College of Pharmacy, old Jewargi Road, Kalaburgi, Karnataka India.⁴Associate Professor, Department of Pharmaceutical Analysis, Anwar ul uloom College of Pharmacy, New Mallepally, Hyderabad, Telangana India⁵Professor, Department of Pharmacognosy, Shadan College of Pharmacy, Peerancheru, Hyderabad, Telangana – India.⁶Assistant Professor, Department of Pharmaceutics, Shadan College of Pharmacy, Peerancheru, Hyderabad, Telangana India.***Corresponding Author: Amjad Pasha**

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ABSTRACT

Celastrus paniculatus is a member of the Celastraceae family and is commonly known as Jyotishmati or black oil plant. *Celastrus paniculatus* has been used extensively in the treatment of a variety of health conditions in traditional medicine. The aim of the present study was to check the presence of phytochemical constituent in ethanolic extract of leaves of *Celastrus paniculatus* using HPTLC analysis. The HPTLC densitometric analysis of the ethanolic extract of leaves of *Celastrus paniculatus* was carried out using CAMAG HPTLC system, and the results were obtained in the form of chromatograms (scanned at the wavelength of 366 nm) representing several peaks. The phytochemical profile of the plant was determined and presented in the tables showing the total number of peaks, peak heights, peak area, percent area, and Rf values.

KEYWORDS: *Celastrus paniculatus*, Ethanolic extract, HPTLC.**INTRODUCTION**

Celastrus paniculatus is a member of the Celastraceae family and is commonly known as Jyotishmati or black oil plant. It is a small to medium sized woody species which is native to India and also widely distributed across countries like Malaysia, Thailand, China, Philippines, North eastern part of Australia.^[1] *Celastrus paniculatus* has been used extensively in the treatment of a variety of health conditions. Various reported activities are antibacterial, antiviral, insecticidal, antispermatogetic, anti-inflammatory, sedative, anti-fatigue and analgesic, hipolipidemic. It is antirhumatic, arthralagenic, aphrodisiac, emetic, laxative, nervine tonic.^[2] *Celastrus paniculatus* is an example of a traditional medication that tribals utilise to treat a variety of ailments.^[3] The bark is a brain tonic, abortifacient, and depurative. The leaves are emmenagogues, and the leaf sap is an effective opium antidote. Celapanin, Celapanigin, Celapagin, Celastrine, and paniculatine are some of the main alkaloids present in the seeds.^[4,5] The leaves include alkaloids, a glycoside, and a bright colouring matter, whilst the oil produced from seeds

contains sterols, alkaloids, and a bright colouring matter. The oil also contains alkaloids and sesquiterpenes such dipalmitoyl glycerol.^[6,7] The aim of the present study was to check the presence of phytochemical constituent in ethanolic extract of leaves of *Celastrus paniculatus* using HPTLC analysis.

MATERIALS AND METHOD

The *Celastrus paniculatus* plant was collected from herbal garden, Himayatsagar Road, Moinabad in the month of December 2018. The plant was authenticated by Botanical survey of India, Deccan regional Centre Hyderabad-500048, Telangana, India.

Preparation of extract with ethanol

Freshly collected leaves of *Celastrus paniculatus* were dried for 12 to 14 days at room temperature. The thoroughly dried leaves were powdered. This powdered plant material was used for extraction. Rinse all the glass apparatus by petroleum ether and dry it in the oven at 102⁰C. Then keep it in desiccator.

Weigh 100gm of powdered leaves of *Celastrus paniculatus* and place it in the thimble in soxhlet extractor. Take a 250ml round bottom flask and clean it and fill the flask with sufficient quantity of ethanol. Place the whole setting on a heating mantle and allow the ethanol to boil. Continue the extraction process for almost 3 to 4 hrs. After removing the condensing unit from extraction unit, allow the sample to cool down. Then the solvent was evaporated and the extract was dried under desiccators. The final extract was then subjected to HPTLC analysis.

High Performance Thin Layer Chromatography (HPTLC):^[8,10]

Instrumentation and chromatographic conditions

The sample was spotted (10 μ L) in the form of band of width of 6 mm with a 100 μ L sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F₂₅₄ (5 cm \times 10 cm) with 250 μ m thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm \times 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 20 cm \times 10 cm twin trough glass chamber using Toluene: Ethyl Acetate: Methanol (7:2:1) v/v as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 380 nm for all developments operated by WINCATS software version 1.4.2.

Method conditions

Made/ Make of Instrument: Desaga Sarstedt Gruppe (Germany).

Development Chamber: 20X10 cm, Twin-trough chamber.

Stationary phase: Pre coated silica gel 60 F₂₅₄ Aluminium plates (Merck, KgaA, Germany).

Plate thickness: 0.2 mm.

Plate size: 200 x 100 mm.

Distance from starting: 20 mm.

Distance from bottom: 10 mm.

Volume applied: 5 μ L.

Table 1: Peak list of Ethanolic extract at UV 366nm.

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	9.8	1917.51	57.89	1063.24	0.01
2	18.8	16.13	0.49	12.83	0.14
3	33.2	19.06	0.58	21.36	0.34
4	54.3	30.68	0.93	13.65	0.63
5	61.7	18.55	0.56	10.72	0.73
6	64.8	145.35	4.39	66.04	0.77
7	71.2	114.39	3.45	41.95	0.86
8	75.7	380.56	11.49	123.4	0.93
9	80	670.01	20.23	317.28	0.99

Band length: 10mm.

Distance between tracks: 20mm.

Development distance: 80mm.

Solvent used: HPLC grade.

Extract storage vials: 5 ml glass vials.

Software: Proquant 1.6 versions.

Mobile phase solvent system: Toluene: Ethyl Acetate: Methanol (7:2:1).

Detection system: UV 366nm.

RESULTS

Chromatogram of the ethanolic extract of leaves of *Celastrus paniculatus*

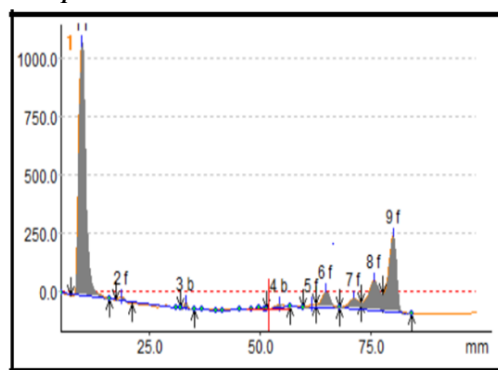


Fig.1: Densitogram of EECP at UV 366nm.

Hptlc Profiling Image of Ethanolic Extract

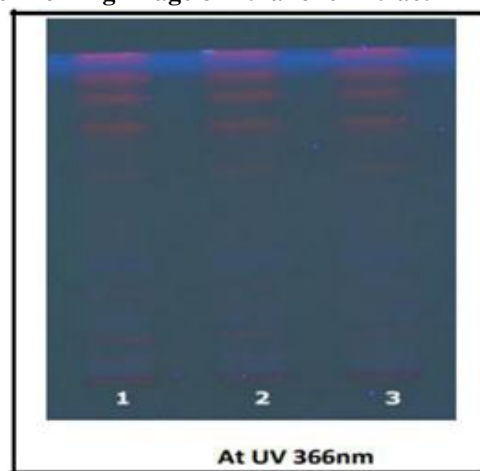


Fig. 2: HPTLC profiling image of Ethanolic extract of *Celastrus paniculatus* leaves. Peak list of Ethanolic extract of *Celastrus paniculatus* at UV 366nm.

DISCUSSION

HPTLC analysis, of ethanolic extract of sample was spotted on silica gel "G" plate and developed with Toluene: Ethyl Acetate: Methanol (7:2:1) as mobile phase shows nine major spots under UV 366nm at Rf values 0.01 (red), 0.14 (light blue), 0.34 (light blue), 0.63 (red), 0.73(red), 0.77(red), 0.86(red), 0.93(red). Component number 9 at 0.99 Rf value showed maximum concentration.

CONCLUSION

In conclusion, the HPTLC method was found to be specific and accurate and can be used for qualitative estimation ethanolic extract of *Celastrus paniculatus* leaves. HPTLC method is especially suitable for the fingerprinting and high throughput analysis of botanical samples.

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