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An HPTLC Fingerprinting Profile Of *Lantana Camara Linn* With Reference To Marker Compound And Phytochemical Screening.

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Abstract:

The current work aimed to create a High-Performance thin layer chromatography (HPTLC) fingerprint profile of *Lantana camara linn* extracts with marker compound (*Lantana camara linn oil)*. For the purpose of separating the components from the various extracts, chromatographic techniques were applied. An HPTLC fingerprint profile of extracts in several solvents including methanol, chloroform, acetone, and Pet.
Ether was intended to be developed in this work The utilization of an automated TLC (Thin-Layer Chromatography) applicator with a solvent mixture comprising Acetic acid: toluene: Ethyl acetate: Methanol (0.5:8:2:0.5 v/v/v/v) was employed for the separation of compounds in the extracts. TLC analysis was conducted on silica gel-coated aluminum plates from Merck. Furthermore, an HPTLC (High-Performance Thin-Layer Chromatography) methodology has been developed for the separation of active components within these extracts. This HPTLC method is suitable for both qualitative and quantitative analysis and can be effectively employed for the standardization of plant extracts. It can be used for routine quality checks of current species.

Keywords: HPTLC, Extraction, Marker compound, Fingerprint

Introduction:

Herbal medicine, a practice as ancient as humanity itself, has been employed for healing purposes throughout history. This enduring connection between humans and the search for natural pharmaceuticals is substantiated by written records, historical monuments, and the original plant remedies. Over countless years of combating diseases, humans have developed an understanding of sourcing pharmaceuticals from various parts of plants, such as bark, seeds, fruit bodies, and more. Consequently, the utilization of therapeutic plants is a wellestablished knowledge. [1,2,3]

Lantana camara Linn. is a popular decorative plant as well as a well-known weed. Plants have been used as a source of medicine since the earliest days of humanity. Long regarded as one of the most important medicinal plants in the world, *Lantana Camara linn* In conventional medicine, *Lantana Camara linn.* is used to treat wounds, swellings, ulcers, cataracts, bilious fever, itches, eczema, and rheumatism. The Lantana camara linn. plant is used in a variety of ways to relieve the symptoms of colds, migraines, coughing, allergies and asthma, chicken pox, pneumonia, eye injuries, and high blood pressure. [4,5]

To identify the main active components of medicinal plants, HPTLC makes sense for the extension of chromatographic fingerprints. In comparison to TLC, the separation and resolution are substantially superior, and the results are far more consistent and repeatable. HPTLC A plant extract or formulation's phytochemical makeup is represented by fingerprinting as a picture at 254nm, 366nm, and white light. It is a series of zones or peaks in a chromatogram that are unique to a given sample. The electronic representation of the visual HPTLC chromatogram is what the United States Pharmacopoeia defines as an HPTLC fingerprint. Based on Rf, color, and the relative intensity of the bands in the electronic image, the HPTLC fingerprint is assessed. [6,7]

For the study of plant active chemicals, many extraction procedures and analytical approaches such as spectrophotometry have been developed [8]. Methods based on high-performance thin layer chromatography (HPTLC) could be a useful alternative, as they are being investigated as a significant tool in regular drug analysis. The capacity of HPTLC to analyze multiple samples simultaneously while employing a modest amount of mobile phase is its primary advantage. This decreases the amount of time and money spent on analysis. Furthermore, it decreases exposure dangers and considerably reduces disposal issues.

Marker compounds are one or more constituents that naturally occur in botanical material and are chosen by a researcher or manufacturer for special attention. The chosen chemicals' concentrations are quantitatively determined in both raw materials and finished goods, and they serve as a guide for product manufacturing.[9]

2. Materials and Methods:

2.1 Collection of Plant Material

The entire *Lantana Camara linn.* plant used in this study was sourced from Sangli district and authenticated by KWC College in Sangli. The fresh plant material was subjected to a thorough cleaning under running tap water, followed by air-drying and subsequent powdering. Lantana camara Linn. oil was employed as a marker compound**.**

2.2 Sample Extraction:

A powdered air-dried drugs weighing about 50 g was extracted sequentially in a Soxhlet device using the following increasing polarity solvents: petroleum ether, acetone, chloroform and methanol. The material was dried before extracting with the next solvent each time. All extracts were concentrated after being filtered with Whatman filter paper. As the sample solution, concentrated extracts were added to the TLC plate.

2.3 Developing solvent system-

Several solvent systems were tested for extract, however the solvent system produced the best results. **Acetic acid : toluene :Ethyl acetate: Methanol (0.5:8:2:0.5 v/v/v/v)**

2.4 Sample application-

Applied bands of each extract using a spray technique, with each band measuring 8mm in length and having a concentration of 0.20 ul for the extract. These samples were duplicated on silica gel 60F_{254}-coated aluminum sheets measuring 100x100mm. To facilitate this process, we utilized the Bandintertrack 5 applicator, which was connected to the CAMAG HPTLC system. The system was controlled by the software version 3.3.33308.1 running on the server DESKTOP-5IHGUM1.

2.5 Development of chromatogram-

Following the application of spots, a chromatogram was developed in a twin trough glass chamber measuring 20x10 cm, which was saturated with a solvent mixture of Acetic acid: toluene: Ethyl acetate: Methanol in a ratio of 0.5:8:2:0.5 v/v/v/v.

2.6 Detection of spots-

The air-dried plates were examined under ultraviolet light until midday. Subsequently, the chromatograms were scanned using a densitometer at a wavelength of 421 nm after being treated with ASR reagent acid. The DESKTOP-5IHGUM1, version 3.3.33308.1, was utilized to record the Rf values and fingerprint data.

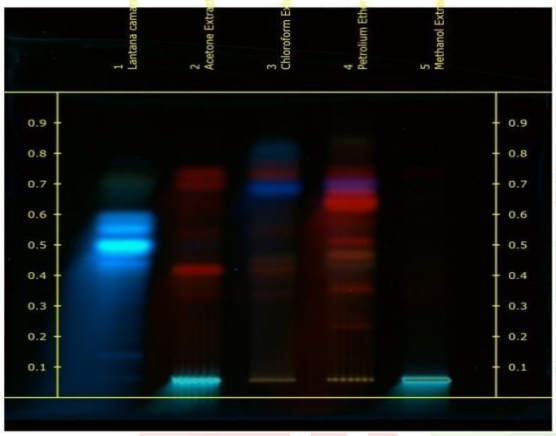

Fig1: HPTLC plate at 254nm

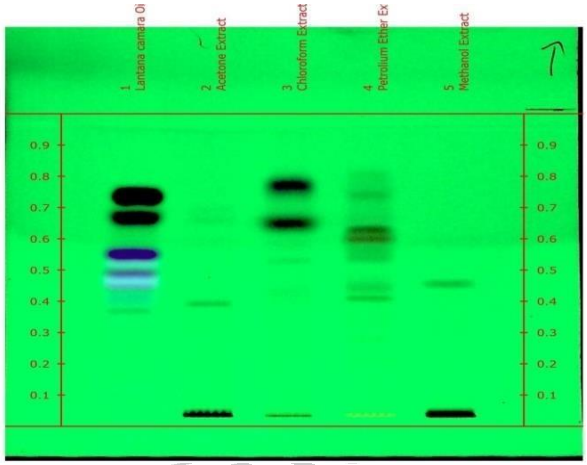

Fig 2: HPTLC plate at 366nm

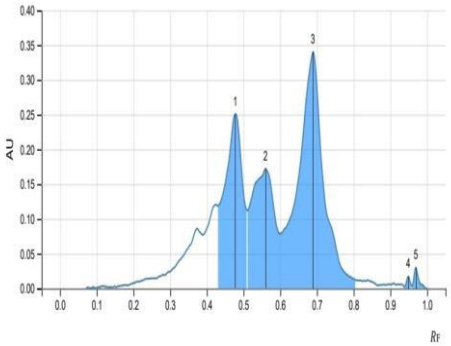

Fig3: HPTLC Chromatogram of Lantana camara L. Camara L.oil at 421nm

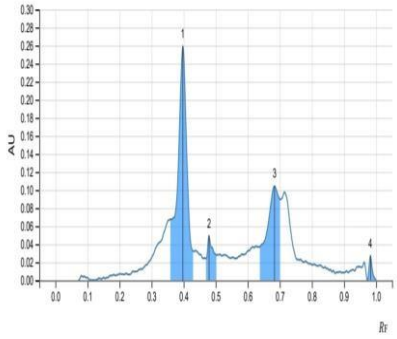

Fig4. HPTLC Chromatogram of Lantana acetone extract at 421nm

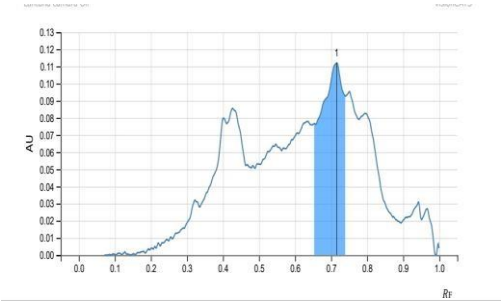

Fig 5:HPTLC chromatogram of Lantana Camara L. L. The chloroform extract 421nm

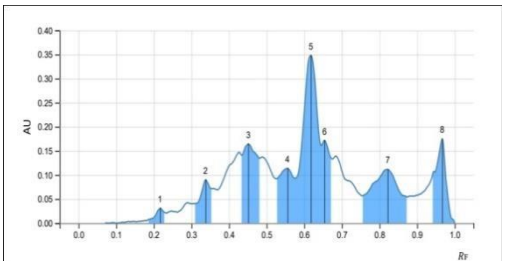

Fig6 :HPTLC chromatogram of Lantana Camara Pet. Ether extract at 421 nm

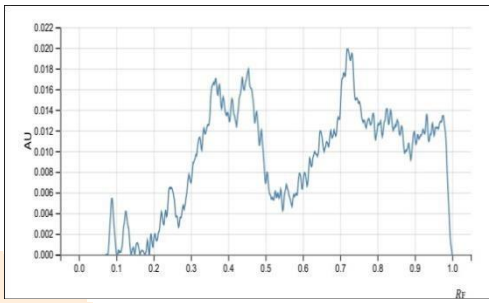

Fig 7:HPTLC chromatogram of Lantana Camara L. Methanol extract at 421 nm

3. Phytochemical Screening-

Chemical tests were carried out for Acetone, pet.Ether, Chloroform, and Methanolic extracts using standard procedures to identify different constituents

The investigation of bioactive substances includes both phytochemical and pharmacological techniques. [10]

Phytochemicals are compounds produced by various plant sections, such as alkaloids, flavonoids, terpenoids, steroids, tannins, glycosides, and so on. Antimicrobial and antibacterial activities of bioactive substances vary the importance of quantitative phytochemical screening in detecting diverse biochemical substances produced by plants is critical. The measurement of these metabolites could help with the extraction, purification, and identification of bioactive substances for human application. Trease and Evans 1989 outline conventional procedures for performing basic qualitative phytochemical screening.[11]

Test for Alkaloids [12]

Wagner's Test: Adding 2-3 ml of extract with a few drops of Wagner's reagent produces a reddish-brown precipitate, indicating the presence of alkaloids.

Dragendorffs Tests: When 2-3 extracts are mixed with a few drops of Dragendorffs reagent, an orange-brown precipitate forms, indicating the presence of alkaloids.

Test for Flavonoids [12]

Shinoda Tests: In a test tube containing 2-3 ml extract, a few fragments of magnesium metal were added, followed by a dropwise addition of concentrated HCl. The formation of a magenta color indicated the presence of flavonoids.

NaOH Tests: In a test tube, a few drops of sodium hydroxide solution were added to 2-3 ml of extract. The appearance of a vivid yellow color that turned colorless upon the addition of a few drops of dilute HCl confirmed the presence of flavonoids.

Test for Glycosides [12]

Keller-Kiliani Test: 2 milliliters of extract were mixed with glacial acetic acid, one drop of 5% FeCl3, and concentrated H2SO4. A reddish-brown color appeared at the junction of the two liquid layers. The upper layer appeared bluish-green which indicates the presence of glycosides.

Concentrate H2SO4 Test: To detect glycosides, add 2ml glacial acetic acid, one drop of 5% FeCl3, and conc. H2SO4 to 5ml extract. A brown ring indicates their presence.

Test for Phenols

Ellagic Acid Test: The test solution was treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) NaNO2 solution. The solution turned muddy, and a brown precipitate occurred in the extract, indicating the presence of phenols. [13]

Test for tannins:[12]

Gelatin Test: To the gelatin solution, warm water was immediately added. The formation of a white precipitate indicated the presence of tannins.

Lead acetate test: To 5 mL of extract, add a few drops of 10% lead acetate solution. The formation of a yellow or red precipitate indicates the presence of tannins.

Tests	Petroleum ether	Chloroform	Acetone	Methanol
Alkaloids				
Wagner's Test	+	+	+	-
Dragendorff Test	+	+	+	+
Flavonoids Shinoda Test NaOH Test	+	+	-	+
	+	+	-	+
Glycosides				
Keller-Killani Test	-	-	-	-
Conc. H2SO4	-	-	-	-
Phenol				
Ellagic Test	+	+	+	+
Phenol Test	+	+	+	+
Tannins				
Gelatin Test	-	-	+	+
Lead acetate test	-	-	+	+

Result:

The study revealed that HPTLC fingerprinting analysis *Lantana camara Linn* performed best in Acetic acid: toluene: Ethyl acetate: Methanol (0.5:8:2:0.5 v/v/v/v). solvent solution. After scanning and visualizing the plates in absorbance mode at 254 nm, 366 nm, and visible light range (400-600 nm after spraying with anisaldehyde sulphuric acid reagent), the most significant outcomes were observed at 421 nm. Figure 1 of the HPTLC demonstrates that all sample components were distinctly separated from each other with no tailing or diffuseness.The presence of five compounds was detected in the oil extract of *Lantana camara Linn* using HPTLC fingerprint scanning at 421 nm. The Rf values were between 0.47 ,0.56,0.68,0.94,0.96. in the acetone extract 4 peaks were obtained between the ranges Rf from 0.47,0.56,0.68, 0.94. The chloroform Extract showed a single peak with 0.71 Rf value. The Pet. ether showed 8 peaks and Rf values are 0.21,0.33,0.45,0.55,0.61,0.65,0.82,0.96. the Methanol extract doesn't show any peak. Chromatogram *Lantana camara linn* oil of shows five peaks with Rf 0.47,0.56,0.68,0.94 at 421 nm out of which acetone extract shows four similar peaks i.e. 0.47,0.56,0.68, 0.94. hence it shows that marker compound i.e. *Lantana camara linn* oil might be present in Acetone extract.

The chromatogram obtained through the selected solvent system and its precise Rf value serve as valuable tools for the standardization of extracts. HPTLC fingerprinting of a plant offers crucial insights into the isolation, purification, characterization, and identification of chemical compounds unique to that species. This aids in the accurate identification and quality control of a specific plant species. The present study furnishes ample data for the identification, standardization, and quality assurance of Lantana Camara Linn, a medicinal plant, and also enhances our understanding of the phytoconstituents present in its ethyl acetate and methanol extracts**.**

Conclusion:

It is crucial to prioritize the development of environmentally friendly pharmaceuticals in today's world. The presence of phytochemicals in Lantana camara, L. suggests that this invasive species could be a promising source of innovative drugs. However, a comprehensive analysis of its phytochemical profile, including both qualitative and quantitative assessments, is necessary to fully understand its potential. It is recommended to investigate the antibacterial, antispasmodic, and anthelminthic properties of Lantana camara, L. Additionally, identifying and characterizing the plant's phytochemicals could lead to new discoveries in pharmaceutical research.[14]

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