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HPTLC Fingerprint Analysis of Methanolic Leaf Extract of *Lantana camara*

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Abstract

Natural product, such as plant extract, either as pure compound or as standardized extract, provides opportunities for new drug discovery. The present study is to evaluate the HPTLC analysis of methanolic extract of *Lantana camara* leaves. The HPTLC analysis results of the current study clearly demonstrated that methanolic leaf extract of *Lantana camara* possess 12 flavonoids and 10 phenolic compounds when compared standards catechin and rutin were respectively. The FT-IR spectra of methanolic leaf extract of *Lantana camara* showed broad phenolic OH band at 3392 cm^{-1}, characteristic –CO stretching at 1634 cm^{-1}, aromating bonding at 1059 cm^{-1}, aromatic stretching at 1634 cm^{-1} and –OH phenolic bending around 1195 & 1455 cm^{-1} when compared to standard quercetin. Therefore it can be concluded that the above mentioned bioactive compounds from the methanolic leaf extract of *Lantana camara* would be useful to find out the novel drugs.

Keywords: Medicinal Plant, *Lantana camara*, bioactive compounds, HPTLC and FT-IR.

1 Introduction

Medicinal plants are due to the presence of bioactive compounds play a very important role in human life for maintaining good health. The uses of medicinal herbs in the treatment of infection are an age-old practice and several natural products are used as treatment of many diseases [1]. The search for a newer source of antibiotics is a global challenge, since many infectious agents are becoming resistant to synthetic drugs. There are thousands of medicinal plants known to have a long history of usage for their curative properties against various diseases and ailments [2]. The use of herbal drugs is once more escalating in the form of Complementary and Alternative Medicine. The World Health Organization has stressed on the need for scientific validity of herbal drugs and ensuring, devising and implementing sound science [3]. Several techniques are available for the qualitative and quantitative estimation of phytochemicals present in plants. Nowadays, the new technology has been made to identify, screen and isolate these active compounds [4].

The HPTLC (High-Performance Thin Layer Chromatography) is an advanced form of TLC as it provides high resolution and much accurate data. It is accepted all over the world as one of the most powerful analytical techniques used for phytochemical and biomedical analysis. It is an inexpensive, simple and rapid method for the estimation of chemical components present in test sample and therefore most widely used by pharmaceutical industries for new drug discovery. *Lantana camara* is well known to cure several diseases and used in various folk medicinal preparations. In last few decades, scientist and researchers around the globe have elaborately studied the chemical composition of whole plant of *Lantana camara* as well as biological activities. These studies established the therapeutic potential of *Lantana camara* in modern medicines and a possible candidate for the drug discovery [5]. The present study was carried out to establish the bioactive compounds present in the methanolic extract of *Lantana camara* leaves by HPTLC and FT–IR analysis.

2 Experimental Sections

2.1. Collection and Identification of plant

The leaves of *Lantana camara* has been collected from Koodapattu, Tirupattur, Tamilnadu and India. The composed leaves were washed thoroughly with distilled water, shade dried, finely powdered. The plant authentication (Reg. no of Certificate no: 4133), Presidency college, Plant Anatomy Research Centre, Chennai. The Fig.1.Shows the plant of *Lantana camara.*

Fig.1. *Lantana camara*

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2.2. Extraction

10g of *Lantana camara* leaf powder +100 ml of Methanol (ME) using Soxhlet apparatus. The collection of leaf extract from the Soxhlet apparatus should be done only after the leaves become exhausted state by losing their constituents. The liquid filtrates obtained are dried at 40±2°C temperature to obtain a gummy concentrate of the crude extract. Finally these extract kept in a suitable vessel with labeling, stored and used for further studies.

2.3. HPTLC Analysis

Separation may result due to adsorption or partition or by both phenomenon depending upon the nature of adsorbents used on plates and solvent system used for development method by Khushboo *et al* [6]. The sample was centrifuged at 3000rpm for 2 minutes and collected the supernatant liquid. This solution was used as test solution for HPTLC analysis. 1µl of the above test solution and 3µl of standard solution was loaded as 6mm and length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber and captured the images at White light, UV 254nm and UV366nm. The developed plate was sprayed with respective spray reagent and dried at 100°C in Hot air oven. The plate was photo-documented at Daylight and UV 366nm using Photo-documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage and scanning was done at 254nm. The Peak table, Peak display and Peak densitogram were noted.

Table 1. Peak table for the Flavonoids of Methanolic leaf extract of *Lanatana camara* by HPTLC

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.45	470.9	19455.1	Flavonoid standard
Sample A	1	0.01	419.3	8038.6	Unknown
Sample A	2	0.05	171.2	4198.1	Flavonoid 1
Sample A	3	0.24	11.2	271.4	Unknown
Sample A	4	0.32	18.4	230.1	Unknown
Sample A	5	0.52	27.2	697.4	Unknown
Sample A	6	0.55	38.2	1718.1	Unknown
Sample A	7	0.62	36.0	525.8	Unknown
Sample A	8	0.66	34.1	723.8	Unknown
Sample A	9	0.72	24.1	598.6	Unknown
Sample A	10	0.83	25.3	843.1	Unknown
Sample A	11	0.92	320.6	8446.7	Unknown
Sample A	12	0.95	273.0	7127.5	Unknown

Table 2. Peak table for the Phenolic compounds of methanolic leaf extract of *Lanatana camara* by HPTLC

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.02	654.2	14980.2	Unknown
Sample A	2	0.11	79.7	2025.2	Phenolic 1
Sample A	3	0.20	80.3	2378.3	Phenolic 2
Sample A	4	0.24	41.4	1030.8	Unknown
Sample A	5	0.33	30.7	911.2	Unknown
Sample A	6	0.44	16.3	105.4	Unknown
Sample A	7	0.59	12.6	72.3	Unknown
Sample A	8	0.70	140.5	6046.7	Phenolic 3
Sample A	9	0.75	67.0	3476.2	Unknown
Sample A	10	0.96	151.7	5845.3	Unknown
STD	1	0.69	414.0	9581.0	Phenolic standard

2.4. FT –IR Analysis

Fourier Transform-Infrared Spectroscopy is very much helpful in examining the peak variation of amino groups and carboxylic groups. Some of the infrared radiation is absorbed by the sample and some of it is passed through the resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample, which corresponds to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. Infrared spectroscopy of Shimadzu Corporation of model IR prestige 21 was used. A drop of each extract was applied on a sodium chloride cell to obtain a thin layer. The cell was mounted on the IR and scanned through the IR region method by Ramírez *et al.*[7].

3 Results and Discussion

3.1. High Performance Thin Layer Chromatrography of methanolic leaf extract of *Lanatana camara*

The methanolic leaf extract of *Lanatana camara* was subjected to HPTLC analysis for the presence of flavonoids and phenolics. First, the flavonoid profile of methanolic

leaf extract of *Lanatana camara* was analysed using catechin as reference standard and 1% ethanolic aluminium chloride reagent used as a spray reagent. The plate was photo-documented at UV 366nm using Photodocumentation (CAMAG REPROSTAR 3) chamber. The methanolic leaf extract of *Lanatana camara* shows the presence of 12 flavonoids. Table 1 and Fig.2.against the standard catechin.

Jain *et al*. [8] reported that quercetin and rutin in *Tephrosia purpurea* (TP) leaves using HPTLC. Chakraborthy and Ghorpade (2019) showed quercetin in *Calendula officinalis* Linn (Asteraceae). Mishra *et al*.[9] also reported quercetin in the methanolic extract of *Phaseolus vulgaris.* The Phenolic profile of methanolic leaf extract of *Lanatana camara* was analysed using rutin as reference standard and 20% Sodium carbonate solution followed by Folin Ciocalteu spray reagent.

The plate was photo-documented at UV366 nm using Photo documentation (CAMAGREPROSTAR 3) chamber. Blue, Brown coloured zone at visible light was observed from the chromatogram, which confirmed the presence of phenolic**s** in the methanolic leaf extract of *Lanatana camara* were observed. The methanolic leaf extract of *Lanatana camara* showed the presence of 10 Phenolic compounds Table 2 and Fig.3. against the standard rutin.

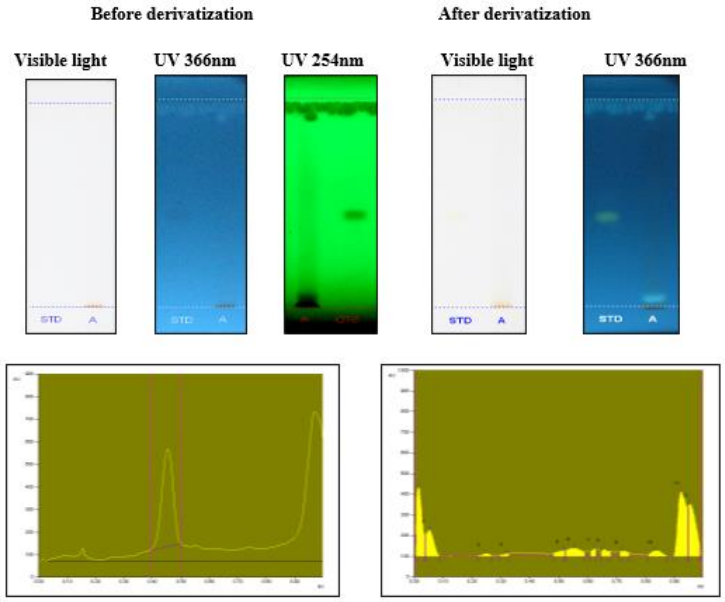

Fig.2. Peak densitogram of Flavonoids of methanolic leaf extract of *Lanatana camara* by HPTLC

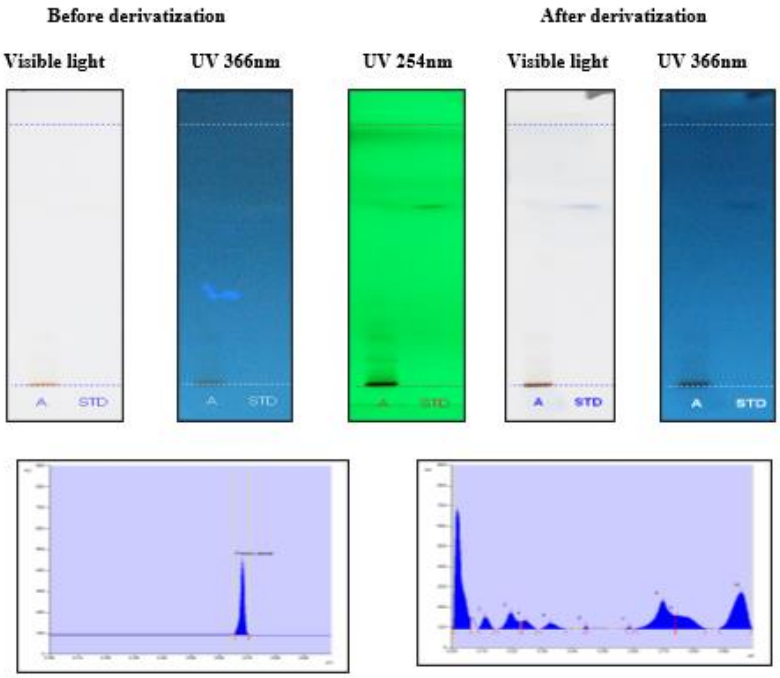

Fig.3. Peak densitogram of Phenolic compounds of methanolic leaf extract of *Lanatana camara* by HPTLC

3.4. FT-IR spectra of of methanolic leaf extract of *Lanatana camara*

The FT-IR spectra of quercetin Fig.4 showed phenolic OH band at 3392cm^{-1}, characteristic –CO stretching at 1654 cm^{-1}, aromatic stretching at 1654 cm^{-1}, aromating bonding at 1025 cm^{-1} and phenolic bending around 1209 &1369 cm^{-1}. The Fig.5 FT-IR spectra of methanolic leaf extract of *Lanatana camara* showed broad phenolic OH band at 3392 cm^{-1}, characteristic –CO stretching at 1634 cm^{-1}, aromating bonding at 1059 cm^{-1}, aromatic stretching at 1634 cm^{-1} and –OH phenolic bending around 1195 & 1455 cm^{-1} were respectively. Similar results for the presence of flavonoids FT-IR spectra were obtained by Sasidharan *et al*. [10] in methanolic crude extract of *Pergularia daemia.*

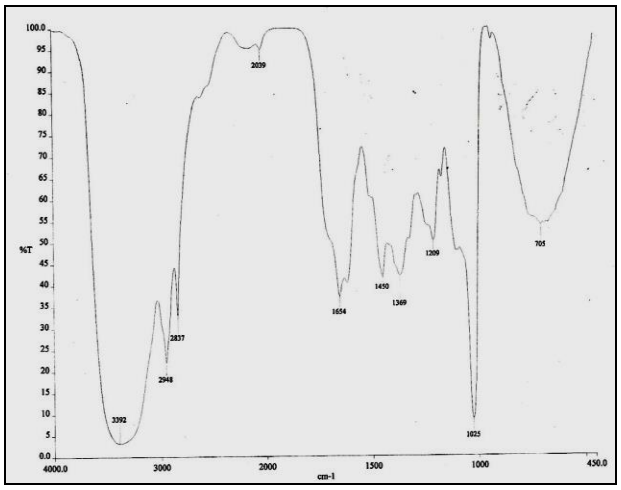

Fig.4. FT-IR spectra of standard quercetin

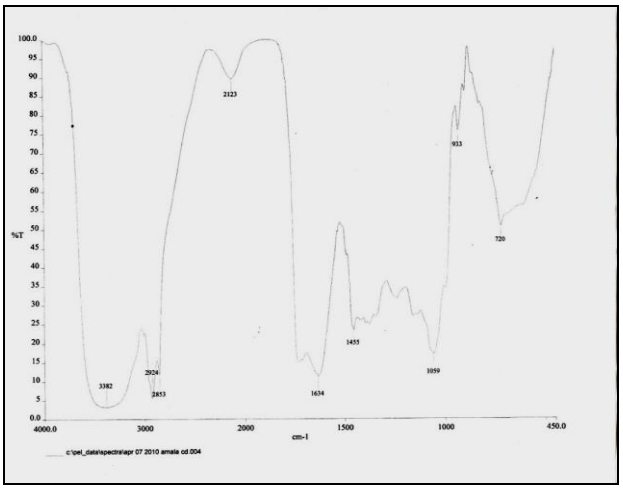

Fig.5. FT-IR spectra of methanolic leaf extract of *Lanatana camara*

4 Conclusions

It can be concluded that the methanolic leaf extract of *Lantana camara* showed HPTLC analysis revealed that the presence of 12 flavonoids and 10 phenolic compounds against the standards like catechin and rutin respectively. The FT-IR spectra of methanolic leaf extract of *Lantana camara* showed broad phenolic OH band, characteristic –CO stretching, aromating bonding, aromatic stretching and –OH phenolic bending when compared to standard quercetin. The results obtained from the current study revealed the presence of various bioactive compounds in the methanolic extract of *Lanatana camara* leaves. The presence of bioactive compounds might be the cause of its healing properties and thus justifies its usage as a remedy in various ailments. New drug formulations require the isolation and identification of important phyto-compounds possessing pharmacological properties. The HPTLC study was carried out for *Lantana camara* chemical profiling will be helpful in the identification of bioactive compounds and markers, by comparing the Rf values of the compounds with the reference standards.

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Conflict of Interest: Nil

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