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HIGH PRESSURE THIN LAYER CHROMATOGRAPHY (HPTLC) FINGERPRINT PROFILE OF EUGENOL FROM *PIPER BETEL* LEAF EXTRACT

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ABSTRACT

The present study was aimed to identify and isolate Eugenol in the methonolic extract of *Piper betel* leaf. HPTLC fingerprinting was carried out for Eugenol in methonolic extract of *Piper betel* leaf. For confirming the presence of Eugenol in *Piper betel* leaf extract, the available standard Eugenol was taken as marker. Presence of Eugenol was checked both at 254nm and 366nm. At 366nm **HPTLC** chromatogram showed presence of Eugenol in the *Piper betel* leaf extract as bluish coloured band (T4), (T5), (T6) at concentrations 4,6, 8 µg/ml. **HPTLC** fingerprinting of both *Piper betel* leaf extract and the standard (Eugenol) was carried out at concentrations 4,6,8 µg/ml. In all the three concentration levels of the *Piper betel* leaf extract, presence of Eugenol was evident.

KEYWORDS: High Pressure Thin Layer Chromatography (HPTLC), Fingerprint Profile, Methanolic Extract and *Piper Betel* Leaf.

INTRODUCTION

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants.**[1]**

By using chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents is not exactly the same for different samples of drug. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic component of the herbal drug.[2-6]

Pharmacognostic study deals with the selection, authentication, collection and quality evaluation of crude drugs and herbal materials based on macroscopic and microscopic characters.[7]

The European Medicines Agency (EMEA) defines chemical markers as chemically defined constituents or groups of constituents of a herbal medicinal product which are of interest for quality control purposes regardless whether they possess any therapeutic activity.

Ideally, chemical markers should be unique components that contribute to the therapeutic effects of a herbal medicine. The study of chemical markers is applicable to many research areas, including authentication of genuine species, search for new resources or substitutes of raw materials, optimization of extraction and purification methods, structure elucidation and purity determination. Systematic investigations using chemical markers may lead to discoveries and development of new drugs.[8]

Physicochemical and phytochemical investigation of plant material and their phytoconstituents plays important role in the field of drug discovery of phytopharmaceuticals.[9]

In many developing countries, a large group of population relies on traditional practioners and medicinal plants to fulfill their primary health care needs.[10]

However, a key impediment, for the worldwide acceptance of the alternative medicines, is lack of documentation and stringent quality control. With this backdrop, it becomes extremely important to make an effort towards systematic standardization of the plant material to be used as medicine.[11]

A large numbers of natural products are being used in the treatment of many diseases as a traditional medicine in several countries. Extracts of *Piper betel* are used for the treatment of various ailments since ages due to its

essential properties like antioxidant, anticancer, anti-allergic etc., *Piper betel* belongs to the family *Piperaceae* and has over 2000 species. The plant is indigenous to India.[12]

Eugenol, one of the principal constituent of *Piper betel* leaf has also been shown to possess anti-inflammatory effects in various animal models of studies with various inflamogens. Mechanistic studies with in vitro systems showed that eugenol blocked the release of the bone resorbing mediators, including IL-1β, TNF-α, and PGE2 from of LPS-stimulated human macrophages by suppressing the messenger RNA expression of LPS-induced IL-1β, TNF-α and COX-2 in macrophages. Eugenol suppressed the COX-2 gene expression in LPS-stimulated mouse macrophage cells.[13]

MATERIAL AND METHODS

Collection of Plant Materials

Piper betel leaves were collected from local market. The leaves were identified and authenticated at Department of Horticulture, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad and the voucher specimen was deposited.

Chemicals

All chemicals and reagents were of analytical grade, purchased from Merck.

Preparation of Extract

The freshly collected *Piper betel* leaves were washed, shadow dried and again dried in hot air oven at temperature not exceeding 50^{o} C. the dried leaves were coarsely powdered mechanically. Powdered leaves 250 gms were then packed in soxhlet apparatus and successively extracted with methanol. The extracts were concentrated in rotary evaporator at temperatures not exceeding 50^{o} C and then dried under vacuum desiccators. The dried extracts were stored.

HPTLC fingerprinting

HPTLC study was carried out on methonolic extract of *Piper betel* leaf. High-Performance Thin-Layer Chromatography was performed on silica gel 60F254 (10 cm× 10 cm; 0.25 mm layer thickness; Merck). *Piper betel* leaf extract was prepared in 10ml volumetric flask by taking 10mg of the *Piper betel* leaf extract and diluting with methanol. The concentration was 1000 µg /ml and filtered through a 0.45 micron syringe filter from this 4,6,8 µg/ml concentrations of each sample were subjected to HPTLC (CAMAG, Switzerland) analysis. All these extract of 3 different concentrations were spotted on a silica gel 60F254 (Merck, Darmstadt, Germany) TLC plate. The plate was air dried and then developed by using the solvent system Hexene: chloroform: Methanol (4:4:2 v/v) as mobile phase in a CAMAG- twin-trough glass chamber previously saturated with mobile phase vapor for 20 min. After developing the plate, it was dried at 65^{o}C for 2 min and then it was scanned using CAMAG Scanner 3 (CAMAG, Switzerland) at 254 and 366 nm using WinCATS 4 software.

Concentration of stock sample (*Piper betel* leaf extract): 1000 µg /ml, Concentration of stock standard (Eugenol): 200 µg /ml.

RESULTS

Eugenol was detected in all the three concentrations of 4, 6, and 8 µg/ml in the *Piper betel* leaf extract in developed TLC plates. This is in accordance with the values obtained with the standard eugenol as marker in the study.

Track1(T1), Track2 (T2) and Track3 (T3) are standard (Eugenol) at 4,6, 8 µg/ml concentrations (both 254nm and 366nm) (figure 1)

Track4 (T4), Track5 (T5) and Track6 (T6) are sample (*Piper betel* leaf extract) 4,6, 8 µg/ml. (both 254nm and 366nm) (figure 2)

At 366nm **HPTLC** Chromatogram showed presence of eugenol in the *Piper betel* leaf extract as bluish coloured band (T4), (T5), (T6) (figure 2)

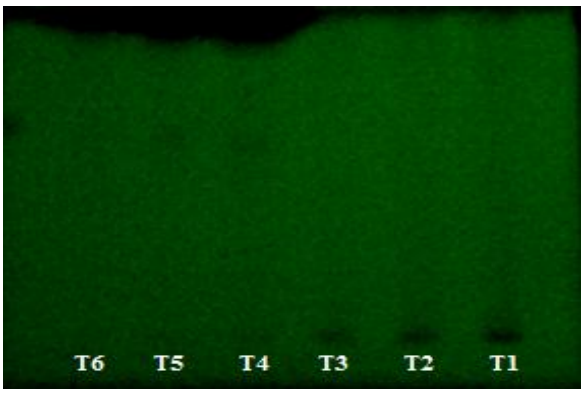

Figure No: 1: HPTLC Chromatogram of Standard (Eugenol) and Sample (*Piper betel* leaf extract) at 254 nm.

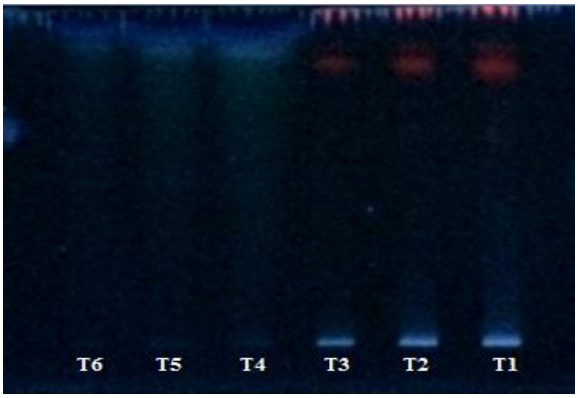

Figure No: 2: HPTLC Chromatogram of Standard (Eugenol) and Sample (*Piper betel* leaf extract) at 366 nm.

HPTLC Analysis

HPTLC fingerprinting of both *Piper betel* leaf extract and the standard (Eugenol) was carried out at concentrations 4,6,8 µg/ml at 254nm and 366nm.

HPTLC densitogram at 254nm showed presence of Eugenol in the *Piper betel* leaf extract at R_f value 0.83 (peak 4, figure 3).

HPTLC densitogram of Eugenol (Standard) at 254nm depicted R_f value 0.89 (peak 4, figure 4)

At 366nm **HPTLC** densitogram showed presence of Eugenol in the *Piper betel* leaf extract at R_f value 0.84 (peak 5, figure 5) At 366nm the R_f value of Eugenol (Standard) was 0.89 (peak 3, figure 6).

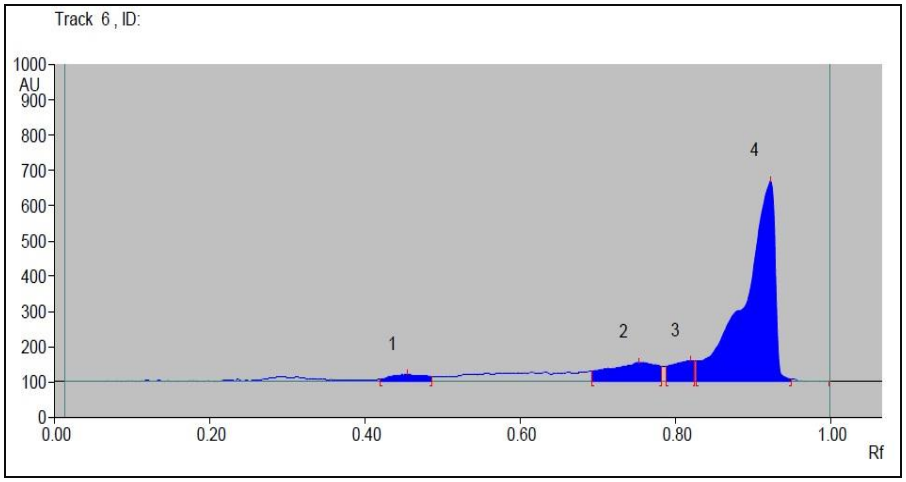

Figure No: 3: HPTLC densitogram of Sample (*Piper betel* leaf extract) R_f value 0.83 at 254nm (Peak- 4).

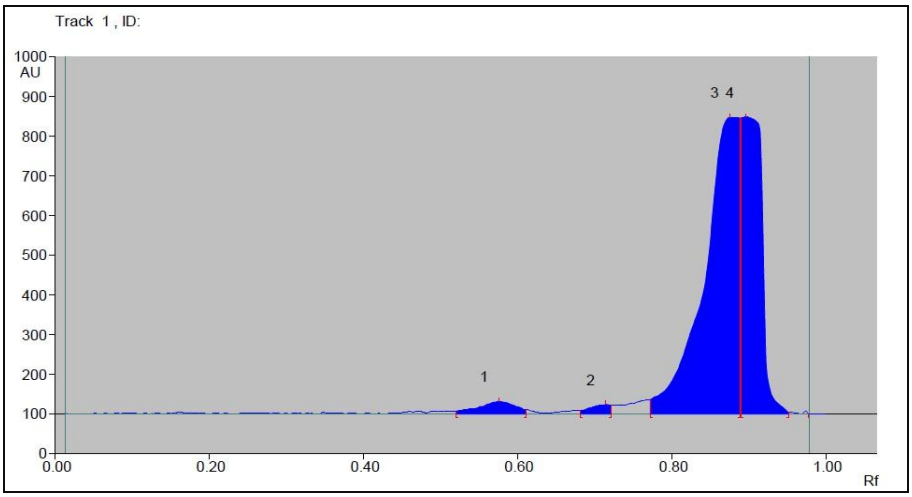

Figure No: 4: HPTLC Densitogram of Standard (Eugenol) R_f value 0.89 at 254 nm (Peak-4).

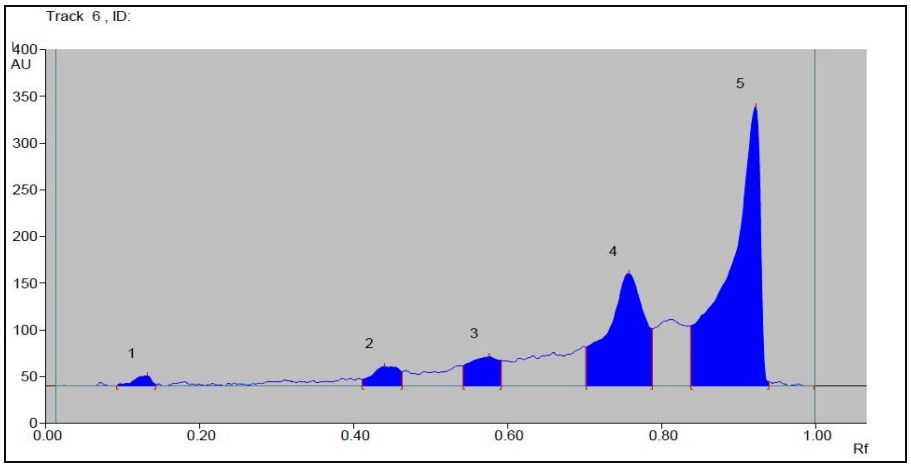

Figure No: 5: HPTLC densitogram of Sample (*Piper betel* leaf extract) R_f value 0.84 at 366nm (Peak- 5).

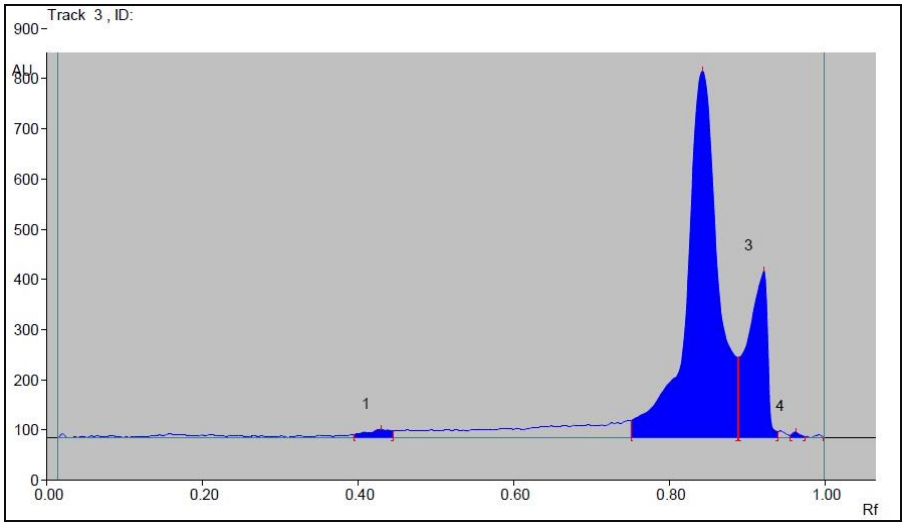

Figure No: 6: HPTLC densitogram of Standard (Eugenol) R_f value 0.895 at 366nm (Peak-3).

DISCUSSION

The greatest impact of plant-derived drugs has been in the anticancer area, wherein the development of plant-derived drugs such as taxol, vinblastine, vincristine, and camptothecin has proven to be a boon in the treatment of some of the deadliest cancers.[14]

The Methanolic Extract of *Piper betel* at 500 mg⁄ kg dose produced immunosuppression that was almost equivalent to that produced by the well-known immunosuppressive drug cyclophosphamide (2 mg⁄ kg), and concluded *Piper betel* a potential candidate for immunomodulatory drug.[15]

HPTLC fingerprinting profile is very important parameter of herbal drug standardization for proper identification of medicinal plants.[1]

In our study HPTLC fingerprinting of *Piper betel* leaf extract showed the presence of Eugenol a phenolic compound. This was substantiated in the HPTLC study carried out both at 254nm and 366nm. HPTLC fingerprinting of both *Piper betel* leaf extract and the standard (Eugenol) was carried out at concentrations 4,6,8 µg/ml. In all the three concentration levels of the *Piper betel* leaf extract, presence of Eugenol was evident.

CONCLUSION

HPTLC fingerprinting is a reliable diagnostic approach in the detection of bioactive compounds.

The study established the presence of Eugenol, a phenolic compound in *Piper betel* leaf extract by HPTLC fingerprinting with the marker compound Eugenol as standard.

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