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High Performance Thin Layer Chromatography Fingerprinting Analysis of *Piper betle* L. Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Many modern medicines used today based on plants and plant products. *Piper betle* is generally known as the betle vine, it is an important medicinal and recreational plant. High performance thin layer chromatography (HPTLC) is an advanced powerful analytical method with more separation power, high performance and superior reproducibility than classic thin layer chromatography (TLC). A chromatographic fingerprint of a plant extract is a chromatographic pattern of some common chemical constituents of pharmacologically active and/or chemical characteristics. Chromatographic fingerprints are useful in authentication and identification of plant.
Objectives: Objectives of present research was to establish HPTLC fingerprinting of methanolic extract of *Piper betle* L. leaves.
Materials and Methods: Methanolic extract of *Piper betle* leaves was prepared using soxhlet apparatus. HPTLC studies were performed using a CAMAG HPTLC system equipped with automatic TLC sampler-4 (ATS 4), TLC scanner 4, and vision CATS 3.0 software.
Results: The study revealed the presence of alkaloids with Rf value 0.65, flavonoids with Rf values 0.19, 0.29, 0.72, 0.95., and phenolic compound with Rf value 0.7.

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Conclusion: The HPTLC fingerprinting profile developed for the methanolic extract of *Piper betle* L. leaves will help in proper identification of the plant.

Keywords: Piper betle; HPTLC; alkaloids; flavonoids; phenolic compounds.

1. INTRODUCTION

The chemistry of plants is divergent as the great variety of forms in which plants occur. The therapeutically important constituents are usually found to be associated with many other substances. Owing to the medicinal properties pertaining to a crude drug, it is important to retain its quality and purity. With the advent of new analytical tools and sophisticated instruments it is possible to suggest a practicable quality assurance profile for a crude drug [1]. HPTLC is a modern powerful analytical technique with high separation power, performance and reprodu cibility superior to classic TLC. A chromate graphic fingerprint of a plant extract is a chromatographic pattern of some common chemical constituents of pharmacologically active and/or chemical characteristics. Chromato graphic fingerprints are useful in authentication and identification of plant [2].

Piper betle Linn. is a perennial dioecious climber, with large leaves, 15-20 cm long, broadly ovate, slightly cordate, shortly acuminate, acute, entire, glabrous, yellowish or bright green, shining on both side [3]. It is generally known as the betle vine, it is an important medicinal and recreational plant [4]. *Piper betle* is extensively found in damp forest and is propagated in India, Vietnam, China and other countries [5]. The leaves of betle vine are nutritive and posses antitumor activity [6], antibacterial [7], antioxidant [8], gastro protective [9], neuroprotective [10], antifilarial [11], anti malarial [12] and analgesic activity [13]. The current investigation aims to analyze the HPTLC fingerprinting profile of alkaloids, flavonoids, phenolic compounds and glycosides for methanolic extract of *Piper betle* L. leaves (MEPBL).

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The leaves of *Piper betle* L. were obtained from the rural areas of Baramati Dist-Pune (Maharashtra) and identified in the Department of Botany, Agricultural Development Trust's Shardabai Pawar Mahila Mahavidyalaya, Sharda nagar Tal-Baramati Dist-Pune, India and was deposited at the college herbarium (Voucher specimen No. PASR-142).

2.2 Preparation of Extract

The fresh leaves of *Piper betle* washed under tap water, air dried, and powdered. Powdered material was subjected to soxhlet extraction with methanol as a solvent. The Extract so obtained was concentrated by evaporating the solvent. The Percent yield was calculated. Extract was stored at room temperature and protected from direct sunlight.

2.3 HPTLC Analysis

2.3.1 Preparation of sample

100 mg MEPBL was dissolved in 5 ml of methanol and solution is sonicated for 15 min and centrifuged for 5 minutes.

2.3.2 Developing solvent system

A number of solvent systems were tried during HPTLC analysis. The satisfactory resolution obtained for the different phytochemical constituent was in the solvent system toluene-ethyl acetate- formic acid (70:30:1), for alkaloid was toluene- ethyl acetate- methanol-ammonia (25%) (30:30:15:1), for flavonoid was ethyl acetate-formic acid-acetic acid-water (100:11: 11:26), for Phenolic compounds was cyclohe xane-ethyl acetate-formic acid (4:6:1) and for the glycoside was ethyl acetate-methanol-water (20:2.8:2).

2.3.4 Chromatography

A 2 µL, 4 µL, 6 µL, 8 µL, 10 µL and 12 µL sample solution were loaded as 8 mm band length on a 100 X 100 mm silica gel $60F_{254}$ TLC aluminum plates using Hamilton syringe and Automatic TLC sampler (ATS 4) instrument.

2.3.5 Development of chromatogram

The samples loaded plate was kept in Twin trough glass chamber 10X10 cm saturated with mobile phase for 20 min.

2.3.6 Detection of spot

The developed plate was dried at room temperature for 5 min., to evaporate solvents from the plate. The images were captured in white light, UV254 nm and UV366 nm. The developed plate was sprayed with Anisaldehyde sulphuric acid reagent for different unknown compounds, Dragendorffs reagent for Alkaloids, Natural product reagent A for Flavonoids, Alcoholic $FeCl_3$ for Glycosides and Alcoholic KOH for Phenolic compounds.

2.3.7 Scanning

After derivatization the plates were scanned in CAMAG TLC Scanner 4. The Peak table, Peak display and Peak densitogram were recorded. Retention factor (R_f), and % area were calculated using vision CATS 3.0 software.

3. RESULTS

HPTLC chromatogram of MEPBL showed different peaks of phytoconstituents. Fig. 1. is the image of TLC plate at 366nm, Fig. 2. is the chromatogram and Table 1. shows the presence of various unknown compounds with retention factor, peak height and peak area.

Presence of alkaloid was confirmed with Rf value 0.65 after derivatization with Dragendorffs reagent as a orange zone is observed. Fig. 3 is the image of TLC plate before derivatization and Fig. 4 is the image of TLC plate derivatized with Dragendorffs reagent captured in white light.

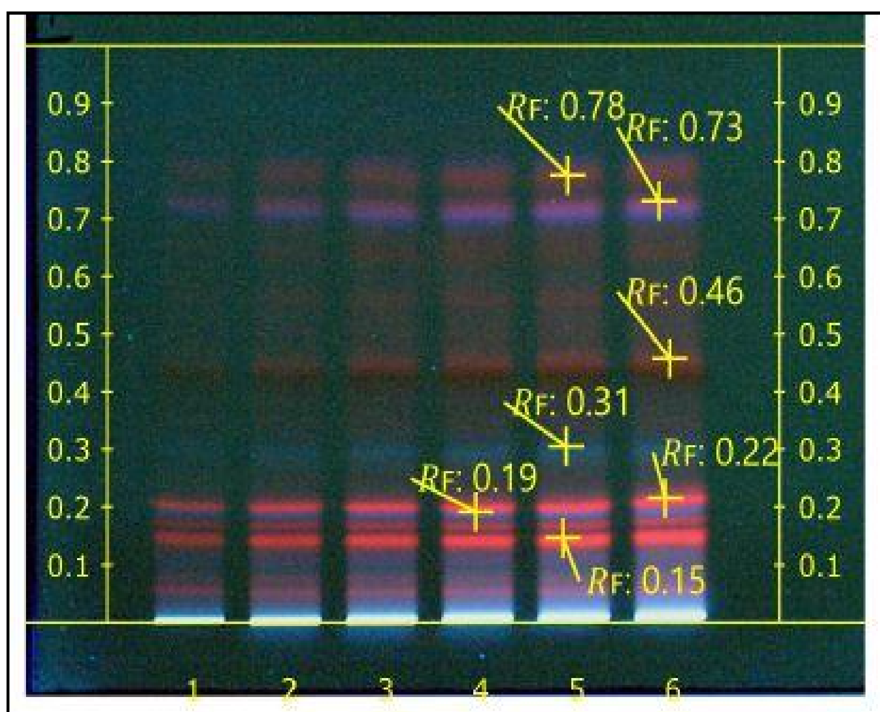

Fig. 1. Image of TLC plate at 366 nm

Table 1. Peak table with Rf values, height and area of unknown compounds

Peak	Rf	Height	Area
1	0.061	0.0162	0.00019
2	0.156	0.2023	0.00400
3	0.182	0.0597	0.00089
4	0.216	0.2124	0.00452
5	0.485	0.0281	0.00040
6	0.724	0.0697	0.00280
7	0.797	0.0275	0.00107

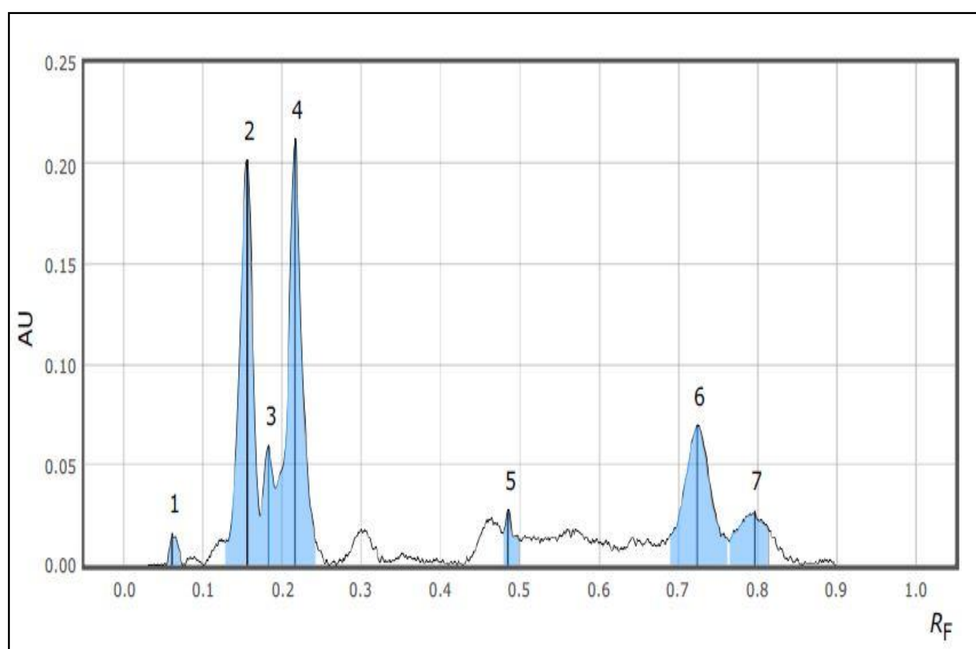

Fig. 2. HPTLC chromatogram of MEPBL at 366 nm

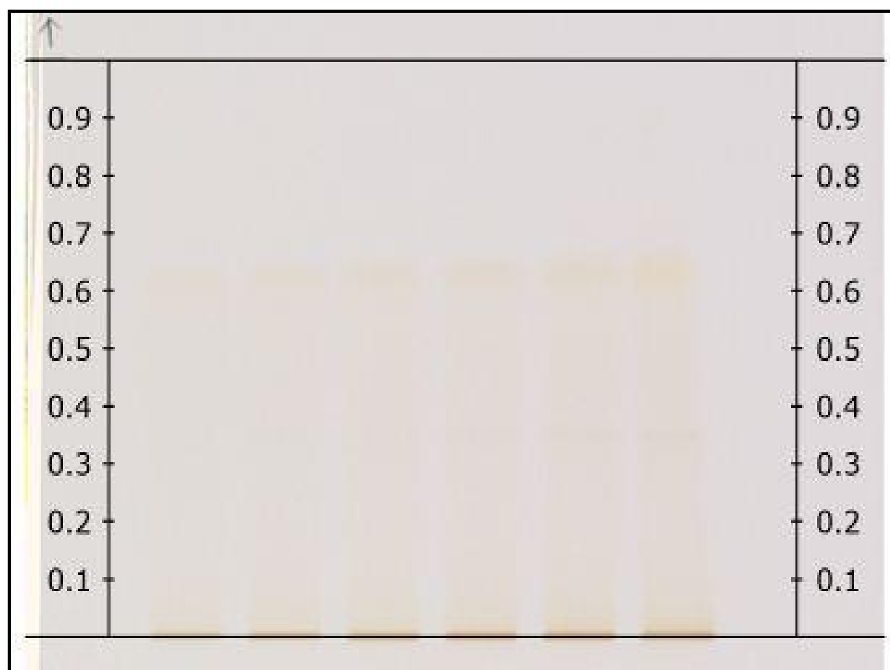

Fig. 3. Image of TLC plate before derivatization in white light

Four flavonoids are detected in MEPBL after derivatization with Natural product reagent A. Rf values was found to be 0.19, 0.29, 0.72 and 0.95. Fig. 5. is the image of TLC plate before derivatization and Fig. 6. is the image of TLC plate derivatized with natural product reagent A captured at 366nm.

Phenols are detected in MEPBL after derivatization with ferric chloride as dark blue

zone (Fig. 7) with Rf value 0.7. However it was not observed before derivatization with ferric chloride (Fig. 8).

Glycosides are not detected as before and after derivatization with alc. KOH dark blue bands are not observed (Fig.9).

4. DISCUSSION

The universal role of plants in the treatment of diseases is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise [14]. In developing countries 80% of the population depends on medicinal plants as a source for their medicine [15]. By considering the demand for the herbs, there is need of simple and rapid analytical method for the plant based medicine. The results from HPTLC fingerprint analysis of MEPBL confirmed the presence of alkaloids with Rf value 0.65, presence of flavonoids are confirmed with Rf values 0.19, 0.29, 0.72 and 0.95 and phenolic compound was confirmed with

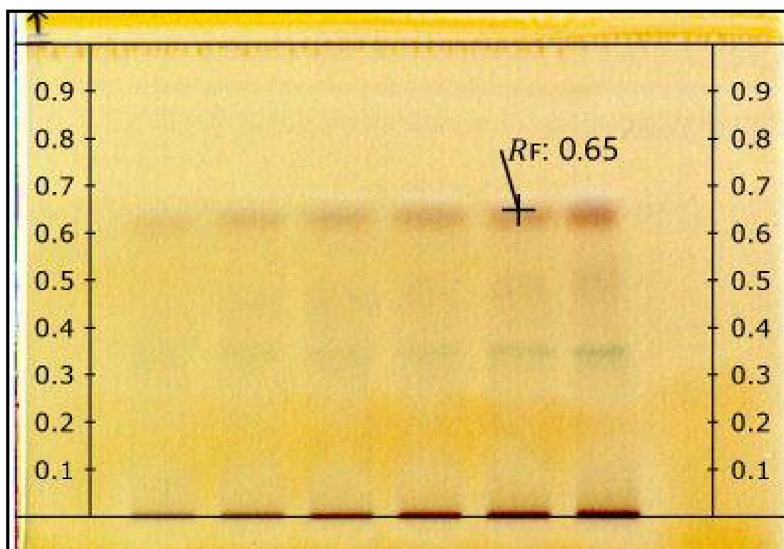

Fig. 4. Image of TLC plate derivatized with dragendorffs reagent captured in white light

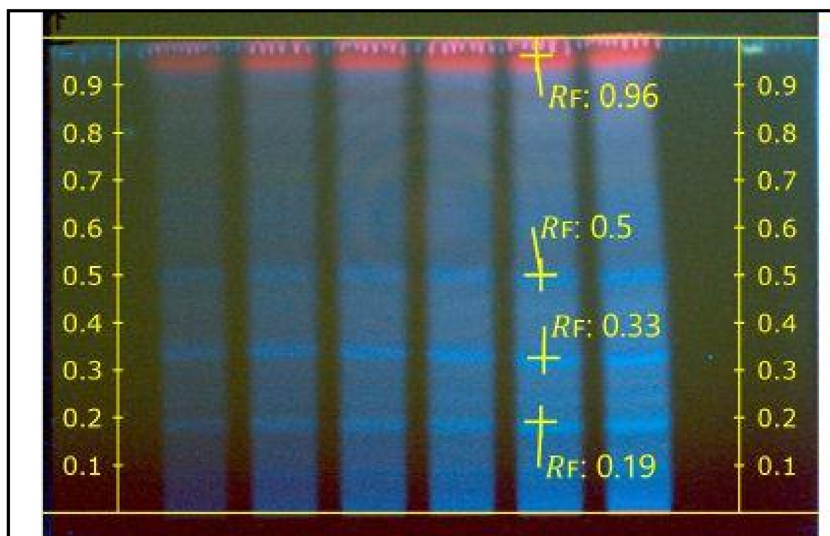

Fig. 5. Image of TLC plate before derivatization captured at 366 nm

Rf value 0.7. However glycoside was found to be absent in MEPBL. HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. A method for HPTLC analysis of *P. betle* has been presented along with results that show the presence of alkaloids, flavonoids and phenolic compounds in the methanolic extract of the plant material.

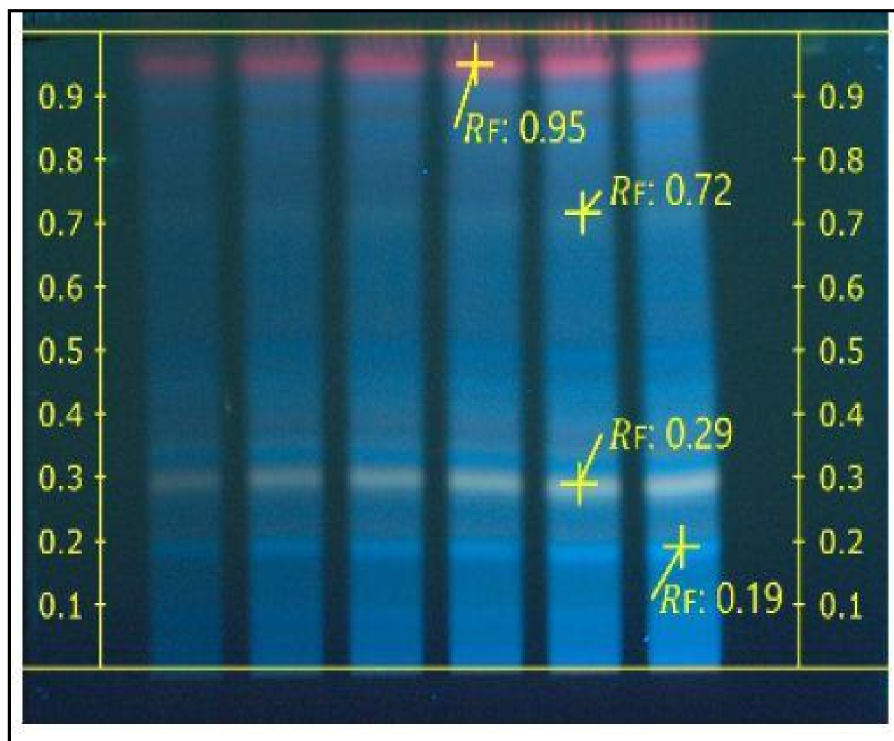

Fig. 6. Image of TLC plate after derivatization with natural product a captured at 366 nm

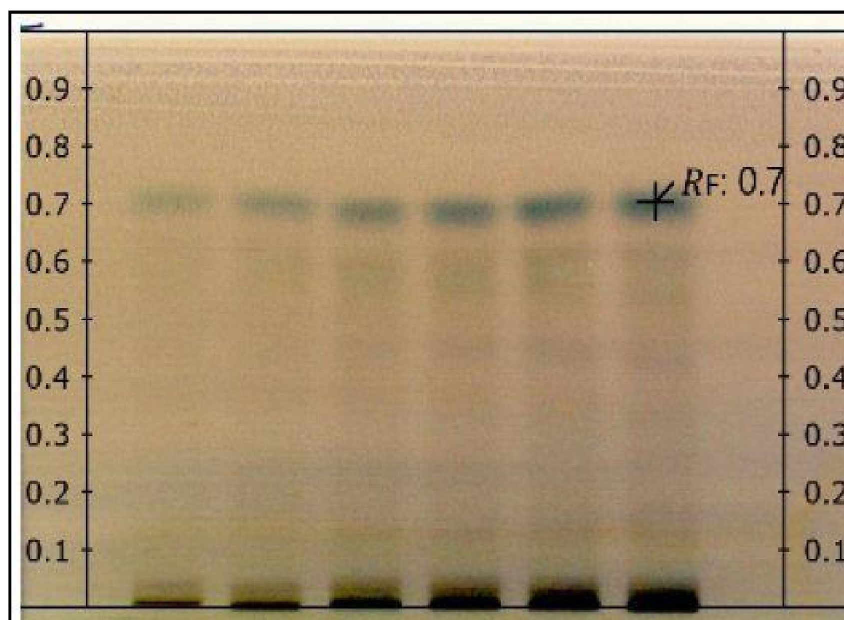

Fig. 7. Image of TLC plate after derivatization with ferric chloride captured in day light

Fig. 8. Image of TLC plate before derivatization with ferric chloride captured in day light

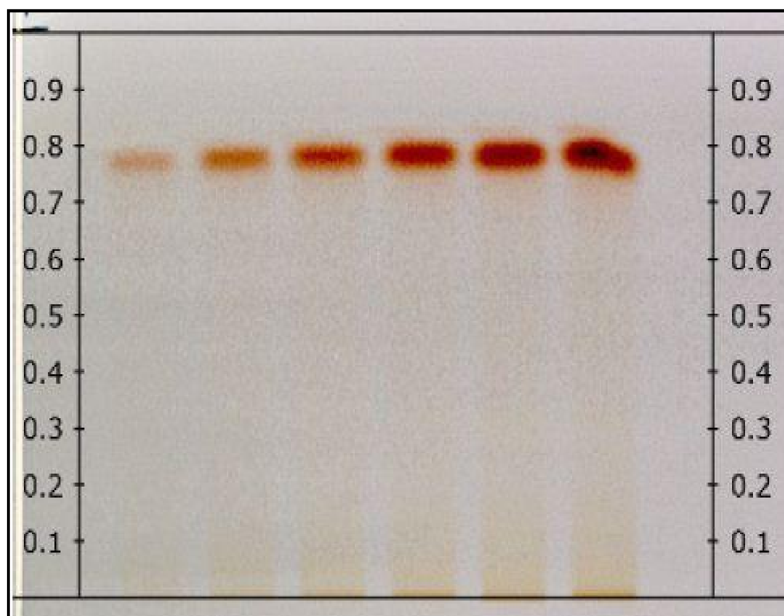

Fig. 9. Image of TLC plate after derivatization with alc KOH captured in day light

5. CONCLUSION

The HPTLC fingerprinting profile developed for the methanolic extract of *Piper betle* L. leaves will help in proper identification of the plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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