

## Research Article

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## HPTLC fingerprinting of extracts of Mango Mistletoe *Helicanthus elastica* (Desr.) Danser with multiple markers

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### Abstract

*Helicanthus elastica* (Desr.) Danser (Loranthaceae) is a less known medicinally important mistletoe species occurring in India for which chemical fingerprinting analysis is wanting. In the present communication, finger print profile of extracts of *H. elastica* has been developed. Chloroform and ethanol extract of the plant were fingerprinted in suitable solvent system and scanned densitometrically under UV and after dipping in vanillin-sulphuric acid reagent followed by heating at 105°C. Presence of different marker compounds in the extract has also been confirmed by co-TLC under suitable solvent system. The total ethanol extract has been fingerprinted using HPLC-UV also. The study revealed differentiating fingerprints which would be helpful in the authentication of *H. elastica*.

**Keywords:** Chemical markers, Chemoprofile, Densitometry, Fingerprinting, Mistletoe.

### Introduction

Chemical fingerprinting will serve as an effective tool in authentication and quality control of herbs. Chromatography can readily ascertain the presence of the essential chemical constituents of a medicinal plant and detection of their presence in a preparation.<sup>1,2</sup> Different chemical fingerprint analysis is currently developed for quality control in Chinese medicine<sup>3-7</sup> and has been accepted by the WHO for the assessment of herbal medicines.<sup>8</sup> The State Food and Drug Administration (SFDA) of China require all herbal medicine-derived injections and related materials to use chromatographic fingerprints<sup>9</sup> in standardization.

HPTLC is being used for fingerprint profiling of medicinal plant extracts since long.<sup>2,10</sup> HPTLC fingerprint profile has been proved to be an effective tool in differentiating closely related species<sup>11</sup> and detecting adulteration and substitution in raw drugs of Indian systems of medicine.<sup>12</sup> The technique is used in standardization of many medicinal raw drugs used in Indian system of medicine.<sup>13-16</sup>

This study has been taken up to derive fingerprint profile of chloroform and ethanol extract of the mistletoe *Helicanthus elastica* by HPTLC in comparison with respective extract of its host *Mangifera indica*. Occurrence of 7 marker compounds isolated from the mistletoe by the authors are assayed by HPTLC.<sup>17</sup>

## Materials and Methods

**Plant material** – Fresh whole plant of *H. elastica* growing on *Mangifera indica* were collected during flowering in the month of August, 2009 from Kasaragod District of Kerala, morphological features were compared with regional floras.<sup>18, 19</sup> It was authenticated by Dr. S. Amerjothy, retired HOD of the Plant Biology and Biotechnology department, Presidency College, Chennai. Voucher specimen (00637) of the plant was deposited at the Pharmacognosy department of Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai. Shade dried powder of whole plant was used for HPTLC fingerprinting. Branches of *M. indica* of same thickness, as that of the branch on which the mistletoe infested, was collected from the same tree, made into pieces and powdered after complete shade drying.

### HPTLC fingerprinting of successive chloroform and ethanol extract

**Extraction** – Successive chloroform and ethanol extracts of *H. elastica* and the host *M. indica* were prepared by Soxhlet extraction of 5 g of air dried plant material. Total ethanolic extract of the mistletoe and the host was used. The isolated compounds were dissolved in suitable solvent.

**HPTLC methodology** – Extracts were applied on a pre-coated silica gel F254 aluminium plates to a band width of 9 mm using Linomat 5 TLC applicator. The plate was developed in a suitable solvent system upto 80 mm and the developed plates were visualized and scanned under UV 254 and 366 nm and under white light at 610 nm after derivatisation in vanillin-sulfuric acid spray reagent. Rf, colour of the spots, densitometric scan and superimposability of densitogram were recorded. Substances were assigned based on self-generated Rf table in case of HPTLC of isolated compounds. Densitometric scans for the compounds were done at wavelength at which optimum absorption was obtained, wherever remarkable not observed under UV, densitometry was performed after derivatisation of the plate in vanillin – sulfuric acid.<sup>10</sup> Extract of *Helicanthus elastica* was compared with the respective extract of its host *Mangifera indica*.

### HPTLC comparison of mistletoe and host extract with marker compounds

Extract of *H. elastica* and its host *M. indica* was compared with the aid of the marker compounds isolated from the mistletoe. Co-TLC of the markers with the extracts of

mistletoe and the host was performed on same plate and densitometrically scanned at suitable wavelength.

## Results and Discussion

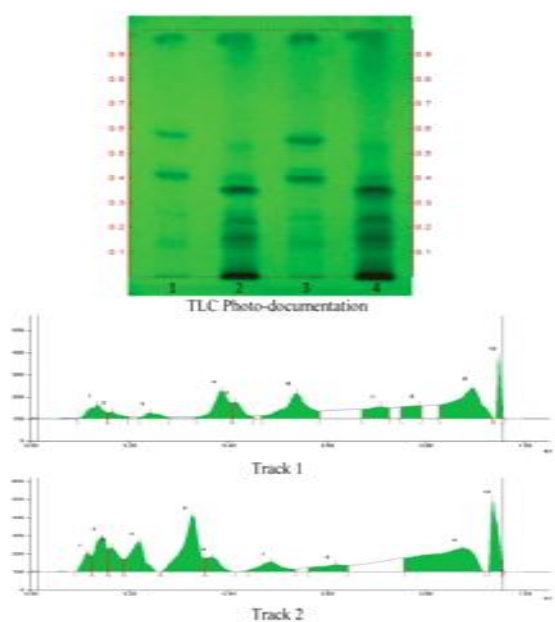
Successive chloroform and ethanol extracts were fingerprinted by HPTLC method in comparison with the respective extract of the host (*M. indica*). The photodocumentation, densitometric scan and Rf value for the extracts in comparison with the respective extracts of the host (*M. indica*) is presented in Figure 1 to 6 and Table 1 to 2. HPTLC of 7 compounds isolated from the plant in comparison with the host extract is presented in Figure 7 to 10.

Chloroform and ethanol extract of both *Helicanthus elastica* and the host plant *Mangifera indica* were compared by HPTLC using suitable solvent systems. When viewed under UV at 254 nm, 366 nm and after derivatization with vanillin-sulphuric acid, good separation of constituents with different Rf values were observed.

The finger print profile of chloroform extract of *H. elastica* under UV light at 254 nm showed 6 spots with Rf values 0.12, 0.14, 0.25, 0.35, 0.41 and 0.56 where as *M. indica* shows 6 spots with Rf values 0.12, 0.16, 0.25, 0.36, 0.40 and 0.52. Two light green spots with Rf values 0.12 and 0.25 were observed in both *H. elastica* and *M. indica*.

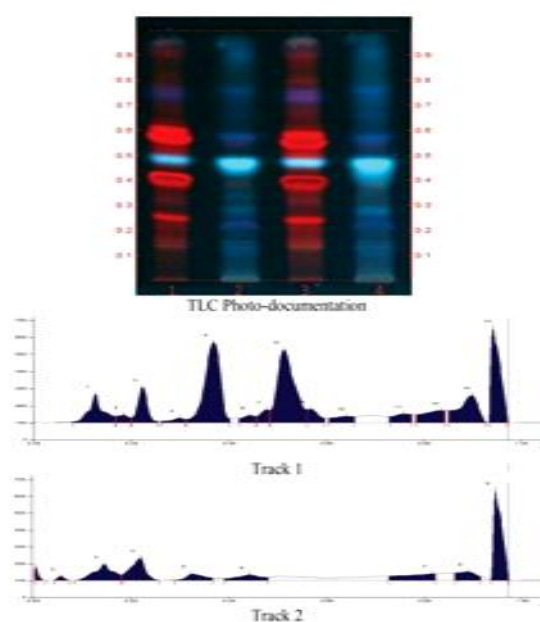
When the plates were view less than 366 nm, the chloroform extract of *H. elastica* showed 16 spots, but *M. indica* showed only 9 spots. The spots with Rf values 0.13 and 0.15 were observed in both *H. elastica* and *M. indica*. In *H. elastica* the colour of the spot at Rf 0.13 is fluorescent brown whereas it was fluorescent blue in *M. indica*. The spot at Rf value 0.15 is fluorescent orange in *H. elastica* but fluorescent green in *M. indica*. Even though the spots with Rf values are same in both *H. elastica* and *M. indica*, the compounds present in both were found to be different as suggested by the difference in colour.

Under white light at 590 nm, the chloroform extract of *H. elastica* showed 9 spots and *M. indica* showed only 6 spots. The two spots with Rf values 0.34 and 0.40 were found common in *H. elastica* and *M. indica*. However the spots at Rf value 0.34 is purple in *H. elastica* and violet in *M. indica*. Whereas the spots at Rf value 0.40 is brown in *H. elastica* and dark blue in *M. indica*.



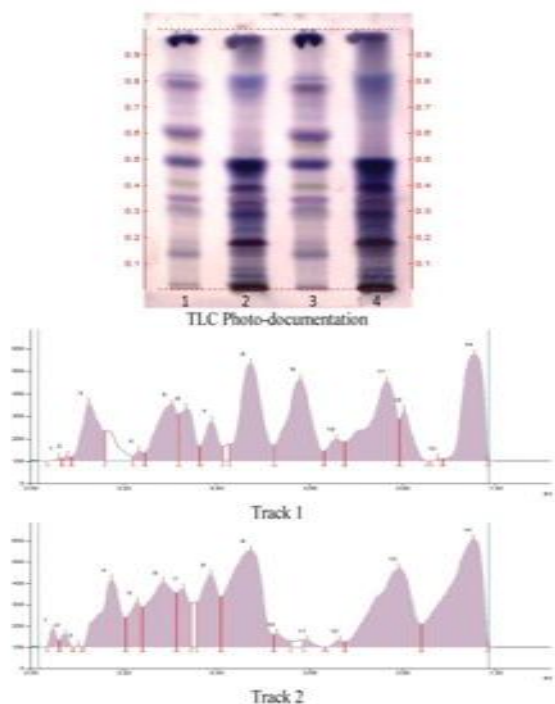
Track – 1& 3 *H. elasticus* 5 & 10 µl; Track – 2 & 4 *Mangifera indica* 5 & 10 µl  
Solvent system : Toluene – Ethyl acetate (10:1.5)

**Fig 1: HPTLC of chloroform extract of *Helicenthus elasticus* under 254 nm**



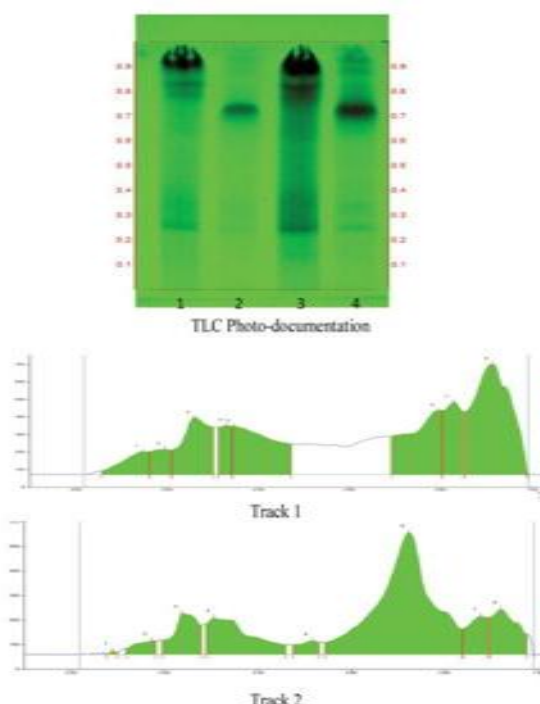
Track – 1& 3 *H. elasticus* 5 & 10 µl; Track – 2 & 4 *Mangifera indica* 5 & 10 µl  
Solvent system : Toluene – Ethyl acetate (10:1.5)

**Fig 2: HPTLC of chloroform extract of *Helicenthus elasticus* Under 366 nm**



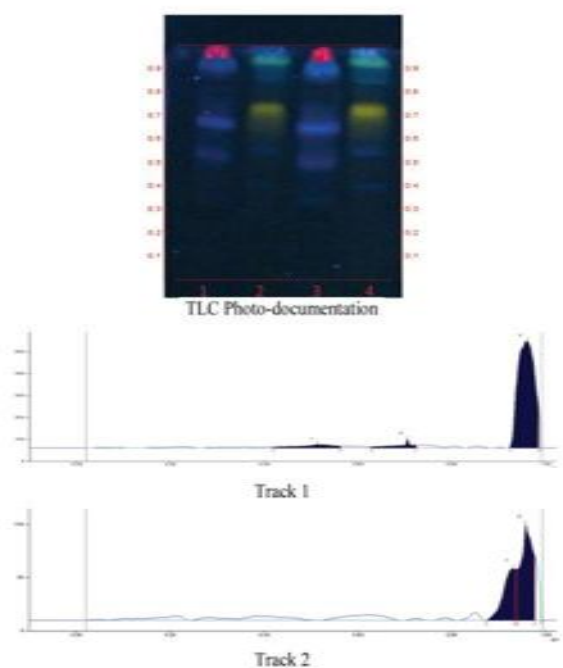
Track – 1& 3 *H. elasticus* 5 & 10 µl; Track – 2 & 4 *Mangifera indica* 5 & 10 µl  
Solvent system : Toluene – Ethyl acetate (10:1.5)

**Fig 3: HPTLC of chloroform extract of *Helicenthus elasticus* after derivatisation**



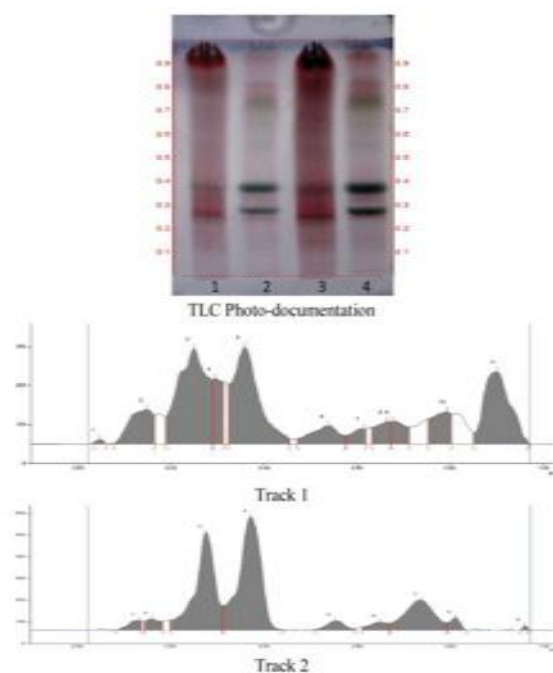
Track – 1& 3 *H. elasticus* 10 & 20 µl; Track – 2 & 4 *Mangifera indica* 10 & 20 µl  
Solvent system : Butanol – Acetic acid – Water (6.3:2.7:1)

**Fig 4: HPTLC of alcohol extract of *Helicenthus elasticus* under 254 nm**



Track - 1 & 3 *H. elasticus* 10 & 20  $\mu$ l; Track - 2 & 4 *Mangifera indica* 10 & 20  $\mu$ l  
Solvent system : Butanol - Acetic acid - Water (6.3:2.7:1)

Fig 5: HPTLC of alcohol extract of *Helicanthus elasticus* under UV 366 nm



Track - 1 & 3 *H. elasticus* 10 & 20  $\mu$ l; Track - 2 & 4 *Mangifera indica* 10 & 20  $\mu$ l  
Solvent system : Butanol - Acetic acid - Water (6.3:2.7:1)

Fig 6: HPTLC of alcohol extract of *Helicanthus elasticus* after derivatisation

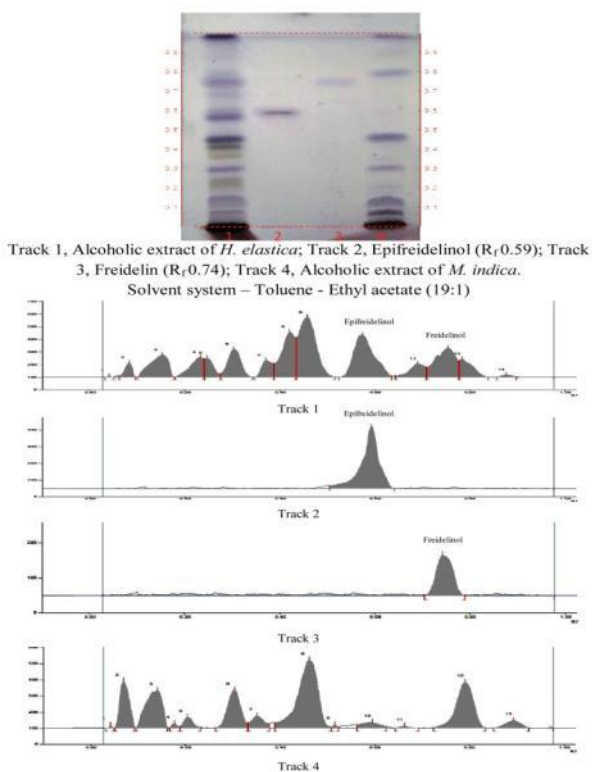


Fig 7: HPTLC of epifriedelinol and friedelin

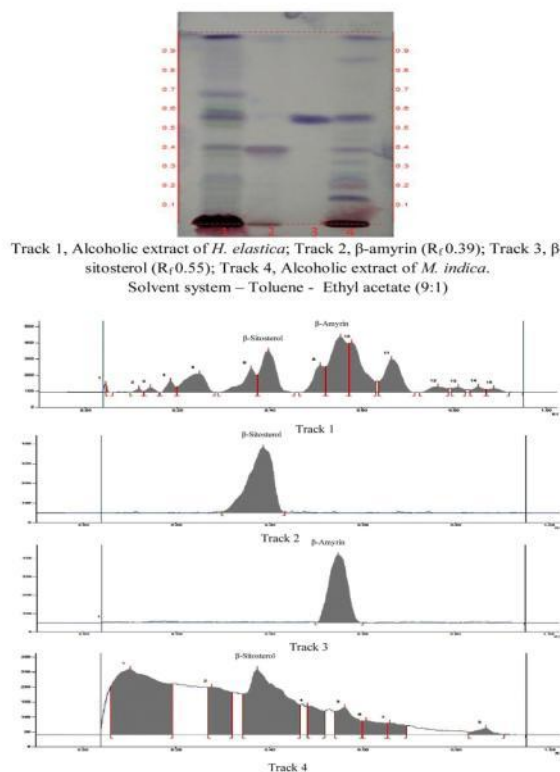


Fig 8: HPTLC of  $\beta$ -amyrin and  $\beta$ -sitosterol

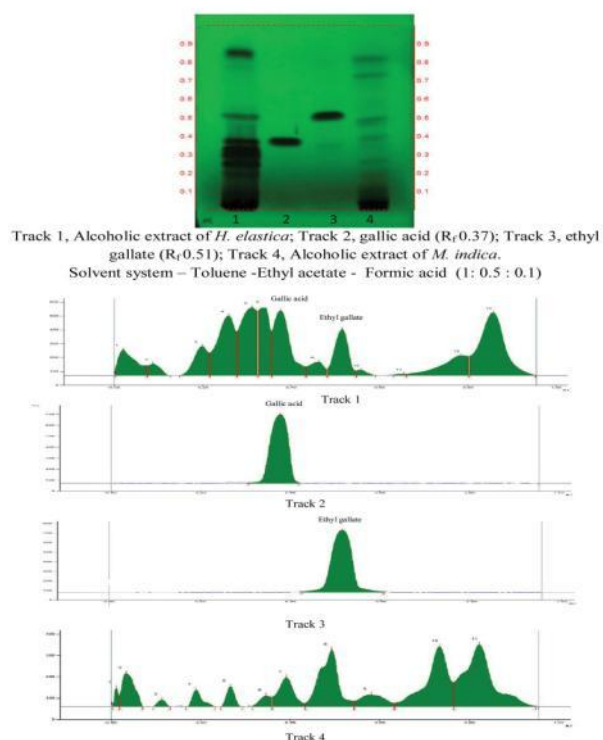


Fig 9: HPTLC of gallic acid and ethyl gallate

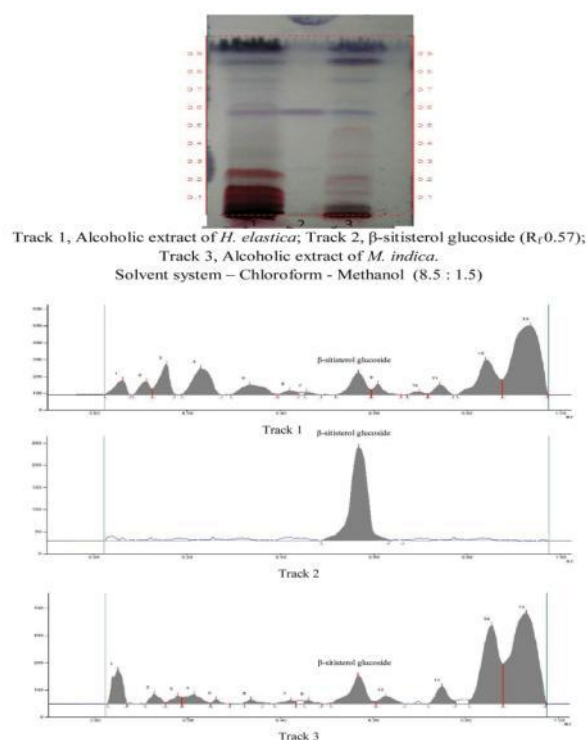


Fig 10: HPTLC of  $\beta$ -sitosterol glucoside

Table 1:  $R_f$  value of chloroform extract of *Helicanthus elastic*

UV 254		UV 366		After derivatisation	
HE	MI	HE	MI	HE	MI
-	-	-	-	-	0.05 blue
-	-	0.08 f. red	-	-	0.08 violet
0.12 l. green	0.12 l. green	-	0.11 f. blue	-	-
0.14 green	-	0.13 f. brown	0.13 f. blue	0.14 blue	-
-	0.16 d. green	0.15 f. orange	0.15 f. green	-	-
-	-	-	0.19 f. blue	0.18 blue	0.19 d. blue
-	-	0.21 f. orange	-	-	-
-	-	0.24 f. red	0.23 f. purple	0.24 brown	-
0.25 l. green	0.25 l. green	-	-	-	-
-	-	-	0.28 f. green	-	-
-	-	-	-	0.30 blue	-
-	-	0.32 f. pink	-	-	-
0.35 d. green	-	-	-	0.34 purple	0.34 violet
-	0.36 d. green	-	-	-	-
-	0.40 l. green	0.39 f. orange	-	0.40.brown	0.40 d. blue
0.41 d. green	-	-	-	-	-
-	-	0.48 f. blue	0.47 f. blue	-	0.48 d. blue
-	-	0.50 f. red	-	-	-

-	0.52 l. green	-	-	-	-
-	-	0.55 f. orange	-	0.55 brown	-
0.56 d. green	-	-	-	-	-
-	-	0.58 f. orange	0.57 f. purple	0.59 violet	-
-	-	0.67 f. red	-	0.66 violet	-
-	-	0.73 f. violet	-	0.72 violet	-
-	-	0.75 f. blue	0.76 f. blue	-	-
-	-	-	-	0.78 violet	-
-	-	0.80 f. red	-	-	-
-	-	0.90 f. pink	-	-	-

d. - dark; f. - fluorescent; HE - *H. elastica*; l. - light; MI - *M. indica*

**Table 2:** Rf value of ethanol extract of *Helicanthus elastic*

UV 254		UV 366		After derivatisation	
HE	MI	HE	MI	HE	MI
0.14 green	-	-	-	-	-
-	-	-	-	0.22 l. green	-
0.24 green	-	-	-	0.24 brown	-
0.25 l. green	0.25 l. green	-	-	0.25 red	0.25 red
-	-	-	-	-	0.28 d. green
-	-	-	-	0.30 blue	-
0.35 d. green	0.33 green	0.34 f. blue	-	0.34 purple	0.34 violet
-	-	-	-	0.37 d. green	0.37 d. green
-	-	0.39 f. orange	-	-	-
0.41d. green	-	-	0.41 f. blue	-	-
-	-	0.50 f. blue	-	-	-
0.56 d. green	-	0.55 f. orange	0.56 f. blue	-	0.55 green
-	-	-	0.57 f. purple	-	-
-	-	0.65 f. blue	-	-	-
-	-	-	-	-	0.72 l. green
-	0.73 green	-	-	-	-
-	-	-	0.74 f. yellow	-	-
0.78 green	-	0.79 f. yellow	-	-	-
-	-	-	-	-	-
0.82 green	-	-	-	-	0.81 l. green
-	-	0.85 f. yellow	0.86 f. green	-	-
-	0.88 green	0.89 f. blue	-	-	-
-	0.92 green	-	-	-	-
-	-	-	0.94 l. f. blue	-	-

d. – dark; f. – fluorescent; HE – *H. elastica*; l. – light; MI – *M. indica*

Ethanol extract of *H. elastica* at 254 nm showed 8 spots but *M. indica* showed only 5 spots. The spot at Rf value 0.25 is light green colour, present in both *H. elastica* and *M. indica*.

Under 366 nm, *H. elastica* showed 8 spots whereas *M. indica* showed only 6 spots. None of the Rf values were found matching to each other.

Under white light at 590 nm, *H. elastica* showed 6 spots and *M. indica* showed 7 spots. The spots at Rf value 0.25 is red in both *H. elastica* and *M. indica*. But the spot at Rf value 0.34 was found to be purple in *H. elastica* and violet in *M. indica*.

Distinct chromatograms were obtained for the chloroform and ethanolic extracts of the plant and also the host.

Rf values of constituents in total ethanolic extract of *H. elastica* and *M. indica* (host) were compared with Rf values of compounds isolated from the plant. Suitable mobile phases for the compounds viz. friedelin, epifriedelinol,  $\beta$ -amyirin,  $\beta$ -sitosterol, gallic acid, and ethyl gallate and  $\beta$ -sitosterol glucoside were used for the HPTLC comparative study.

The marker compound epifriedelinol showed Rf value at 0.59 (purple) and friedelin 0.74 (light purple) in toluene: ethyl acetate (19:1) after derivatization with vanillin-sulphuric acid. Rf values corresponding to friedelin and epifriedelinol were not seen in ethanol extract of *M. indica*

The marker compounds  $\beta$ -amyirin shows Rf value 0.39 (purple) and  $\beta$ -sitosterol 0.55 (dark blue) in toluene: ethyl acetate (9:1) after derivatization with vanillin-sulphuric acid. *M. indica* showed corresponding spot for  $\beta$ -amyirin at the same Rf value with same colour indicates the presence of  $\beta$ -amyirin in *M. indica*.

Rf value of gallic acid was 0.37 (dark green) and ethyl gallate was 0.51 (dark green) in toluene: ethyl acetate: formic acid (1:0.5:0.1) at 254 nm. Ethanol extract of *M. indica* showed the corresponding Rf values for gallic acid but not ethyl gallate.

The marker compound  $\beta$ -sitosterol glucoside showed spot at Rf value of 0.57 (purple) in chloroform: methanol (8.5:1.5) after derivatization with vanillin-sulphuric. Spot with Rf value of  $\beta$ -sitosterol glucoside was present in ethanol extract of *M. indica*, but colour of the spot was blue. Of the isolated compounds, however, all the compounds except ethyl gallate and  $\beta$ -sitosterol were found in both plant and the host.

## Conclusion

The fingerprint would be use in day to analysis of the mistletoe extract with co-injection of analytical marker compounds of the mistletoe. The fingerprinting can also be used to differentiate the mistletoe obtained from different host species. The fingerprint profile obtained from the present investigation can be used for identification and quality control of the extracts of the *Helicanthus elastica* growing on *Mangifera indica*.

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