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HPTLC analysis of coumarin profile in the leaf and bark samples of *Loranthus longiflorus* Desr. (Syn.–*Dendrophthoe falcata* (L.f.) Ettingsh) collected from two host trees

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Abstract
Coumarin compounds profile was determined by HPTLC in the methanol extract of leaf and bark samples of *Loranthus longiflorus* collected from two host trees– *Casuarina equisetifolia* and *Ficus religiosa* and compared. The methanol extract of *Loranthus* leaf samples obtained from *Casuarina* host trees showed ten compounds while it was nine compounds in the leaf samples collected from *Ficus* host tree and were compared with coumarin standard. Among the compounds, 3 and 2 compounds in each sample, respectively was identified as coumarin while all other compounds were unknown. Two compounds from each leaf samples collected from *Casuarina equisetifolia* (peak no. 4 & 10) and *Ficus religiosa* (peak no. 1 & 9) host trees showed similar R_f values (0.22 & 0.94, respectively). On the other hand, the methanol extract of *Loranthus longiflorus* bark sample collected from *Casuarina equisetifolia* and *Ficus religiosa* host trees contained 9 and 4 compounds in each sample, respectively, and were compared with coumarin standard. All these compounds were unknown. Among the compounds, 3 compounds from both bark samples obtained from *Casuarina* equisetifolia (peak no. 1, 4, & 6) and *Ficus religiosa* (peak no. 1, 3, & 4) showed similar R_f values (0.04, 0.36 & 0.52). None of the compounds from leaf and park samples of *Loranthus longiflorus* collected from *Casuarina equisetifolia* and *Ficus religiosa* showed similar R_f values.

Keywords: Coumarin, Leaf, bark, methanol extracts, *Loranthus longiflorus,* Hemiparasite, *Casuarina equisetifolia* host, *Ficus religiosa* host

1. Introduction
Coumarin is a fragrant chemical compound found naturally in many plants and used in the pharmaceutical industry as a precursor molecule in the synthesis of a number of synthetic anticoagulant pharmaceuticals. It has appetite suppressing properties and has evidence of biological activities. Tseng [1] reported that coumarin is a potential antioxidant and its antioxidant activity is due to its ability to scavenge free radicals and to chelate metal ions. Plants containing coumarins are known to exert a beneficial action on immune system by increasing body strength and it can also be suggested to be beneficial for hyperproliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects [2]. It has also been found that coumarin and its derivatives shows wide range of biological activities such as in the treatment of lymphedema[3]; anti-HIV, anti-tumor; anti-hypertension, anti-arrhythmia, anti-osteoporosis, antiseptic, and analgesic; asthma [4]; and anticoagulant, anti-inflammatory, antifungal, anti-ulcer, antimicrobial, vasodilator, estrogenic, etc. [5].
Loranthus species, semiparasitic mistletoe plants, are known to produce a variety of bioactive compounds. *Loranthus longiflorus* possesses remarkable potentials as a medicinal plant evident from the wound healing, anti-microbial, anti-oxidant, antinociceptive properties of its ethanol extracts [6-8]. Medicinal properties of this hemiparasite may vary in effects respective to different hosts and it establishes a relation with bioactivities [9, 10]. The whole plant is used in indigenous system of medicine as cooling, bitter, astringent, aphrodisiac, narcotic and diuretic [11] and is useful in treating pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesical calculi and vitiated conditions of kapha and pitta [12, 13]. The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the coumarin compound profile in the leaf/bark samples of *Loranthus longiflorus*, a hemiparasite.

2. Materials and Methods

2.1 Plant Material

The leaf and bark samples of *L. longiflorus* were collected from two different host trees –*C. equisetifolia* and *F. religiosa*, during July, 2009 to September, 2009 from Nagercoil town area.

2.2 Preparation of plant material powder

Fresh leaf and bark samples of *L. longiflorus* were collected from *C. equisetifolia* and *F. religiosa* host trees and dried separately at room temperature (30 °C±2 °C) for about two weeks to get a constant weight. The dried plant materials (leaf and bark) were ground to powder by mechanical device and stored for further biochemical analysis.

2.3 Preparation of extract

The dried plant materials of *L. longiflorus* leaf/bark samples (5g) from *C. equisetifolia* and *F. religiosa* host trees were extracted with Methanol in soxhlet apparatus for 3hrs. The extract was cooled, filtered and concentrated using a vacuum flask evaporator. Finally this extract was dissolved in 1ml methanol and centrifuged at 3000rpm for 5min. This methanol extract solution was used as test solution for HPTLC analysis.

2.4 HPTLC Analysis

Methanol extracts of *L. longiflorus* leaf and bark samples collected from *C. equisetifolia* and *F. religiosa* host trees were subjected to HPTLC analysis to assess the presence of various coumarin compounds.

2.5 Sample loading: About 3µl of the methanol test solution and 2µl of standard solution (1mg in 1ml methanol) were loaded as 5mm band length in the 3 x 10 silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

2.6 Spot development: The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase and the plate was developed in the respective mobile phase up to 90mm.

2.7 Photo-documentation: The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured at white light, UV 254nm and UV366nm or 500nm.

2.8 Derivatization: The developed plate was sprayed with respective spray reagent and dried at 100 °C in hot air oven. The plate was photo-documented at day light and UV 254nm/UV 366nm, using photo-documentation (CAMAG REPROSTAR 3) chamber.

2.9 Scanning: Before derivatization, the plate was fixed in scanner stage and scanning was done at UV 254nm/ UV 366nm/ UV 500nm. The peak table, peak display and peak densitogram were noted [14].

2.10 HPTLC analysis for coumarin

- **Test solution:** Methanol extracts of *L. longiflorus* leaf/bark samples obtained from *Casuarina* and *Ficus* host trees.
- **Standard solution:** Methanol.
- ***Standard chemical***: COU –Coumarin was used as reference standard compound.
- **Mobile phase:** Toluene-ether (1: 1) saturated with 10% acetic acid.
- **Spray reagent:** 5% Ethanolic potassium hydroxide reagent.

3. Results and Discussion

Coumarin compounds profile in the methanol extracts of *L. longiflorus* leaf and bark samples collected from *C. equisetifolia* and *F. religiosa* host trees were done by HPTLC analysis. The chromatogram (Fig.-1a & 3a) shows coumarin profile of methanol extract of *L. longiflorus* leaf (X) and bark (Y) samples collected from *C. equisetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees and are compared with coumarin (COU) standard. Blue, blue-green, yellow, violet and brown coloured fluorescent zones present in the coumarin standard and plant samples track at UV 366nm mode were observed in the chromatogram after derivatization and this confirmed the presence of coumarin compounds in the leaf and bark samples of *L. longiflorus* collected from two host trees.

HPTLC analysis for coumarin profile in the methanol extract of *L. longiflorus* leaf and bark samples collected from *C. equisetifolia* and *F. religiosa* host trees showed several peaks (R_f -values) of compounds (Tab. 1&2; Fig. 1b&3b) and were compared with coumarin standard (Fig.-1b-iii; Fig.-3b-iii). The densitogram (Fig. 1b&3b) showing the profile of coumarin compounds present in the methanol extract of *L. longiflorus* leaf (X) and bark (Y) samples collected from *C. equisetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees; and coumarin standard for leaf (Fig.-1b-iii) and bark (Fig.-3b-iii) samples scanned at 366nm.

3D display of densitogram for coumarin profile (Fig.-2 & 4) shows all tracks of *L. longiflorus* plant samples (X1/X2-leaf and Y1/Y2-bark) collected from *C. equisetifolia* (X1&Y1) and *F. religiosa* (X2/Y2) host trees and coumarin standard scanned at 366nm.

The methanol extract of *L. longiflorus* leaf samples (X1) obtained from *C. equisetifolia* host trees showed 10 compounds (Tab.-1X1; Fig.-1b-i) with peak R_f values ranging from 0.06 to 0.94, peak height ranging from 13.4 to 184.3 and peak area ranging from 348.1 to 2770.7 as compared to coumarin standard (0.64, 322.7 and 18563.4, respectively). Among the 10 compounds detected, 3 were identified as coumarin (peak no. 2, 9 & 10) and the others were unknown.

On the other hand, the methanol extract of *L. loranthus longiflorus* leaf sample (X2) collected from *F. religiosa* host tree showed 9 compounds (Tab.-1X2; Fig.-1b-ii) with peak R_f values ranging from (0.22 to 0.94, peak height from 12.2 to 50.6 and peak area from 184.9 to 2902.4 as compared to cryptoxanthin standard (0.64, 322.7 and 18563.4, respectively) and out of 9 compounds, 2 were identified as coumarin and others were unknown.

The methanol extract of *L. longiflorus* bark samples (Y1) collected from *C. equisetifolia* host tree showed 9 compounds (Tab.-2Y1; Fig.-3b-i) with varied peak R_f values (0.04-0.96), peak height (10.4-72.2) and peak area (101.4-2420.8) as compared to coumarin standard (0.64, 204.7 and 9015.4, respectively). All the 9 compounds detected were unknown.

Similarly, the methanol extract of *L. longiflorus* bark sample (Y2) collected from *F. religiosa* host tree revealed 4 compounds (Tab.-2Y2; Fig.-3b-ii) with peak R_f values ranging from 0.04 to 0.52, peak height from 14.8 to 49.4 and peak area from 365.7 to 1997.2 as compared to standard coumarin (0.64, 204.7 and 9015.4, respectively). All the four compounds detected were unknown.

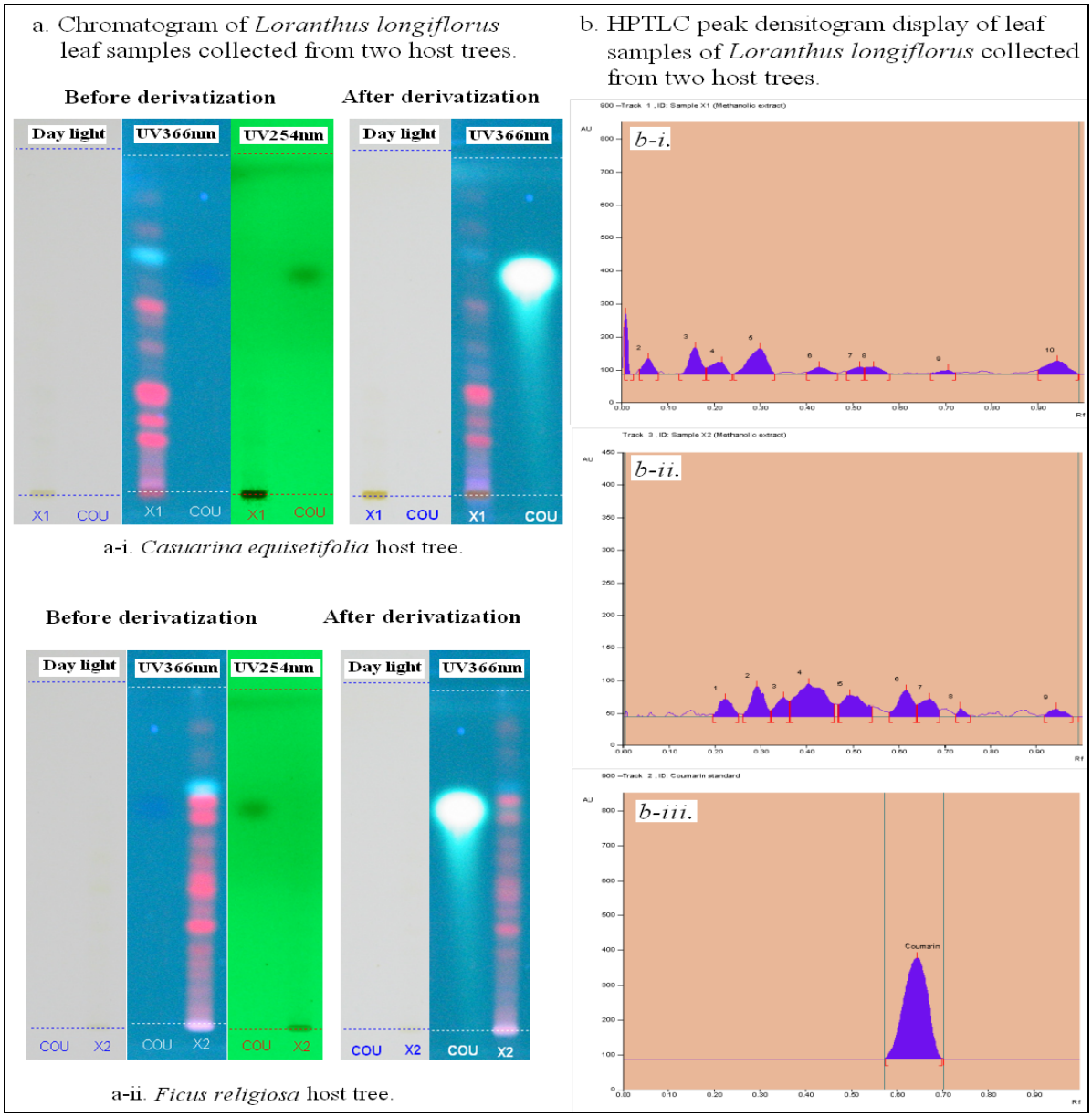

Fig 1: Chromatogram (a) and peak densitogram (b) shows coumarin profile in the *Loranthus longiflorus* leaf samples collected from *Casuarina equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; COU-Coumarin standard -b-iii).

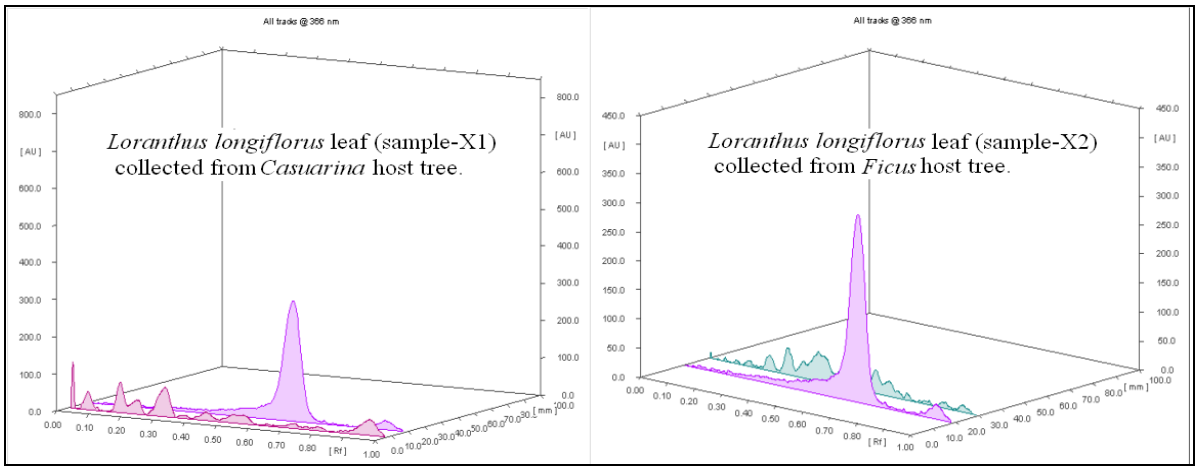

Fig 2: HPTLC-3D display of densitogram showing all tracks –*Loranthus longiflorus* leaf samples (X1/ X2) and standard (Coumarin-pink coloured) scanned at 366nm.

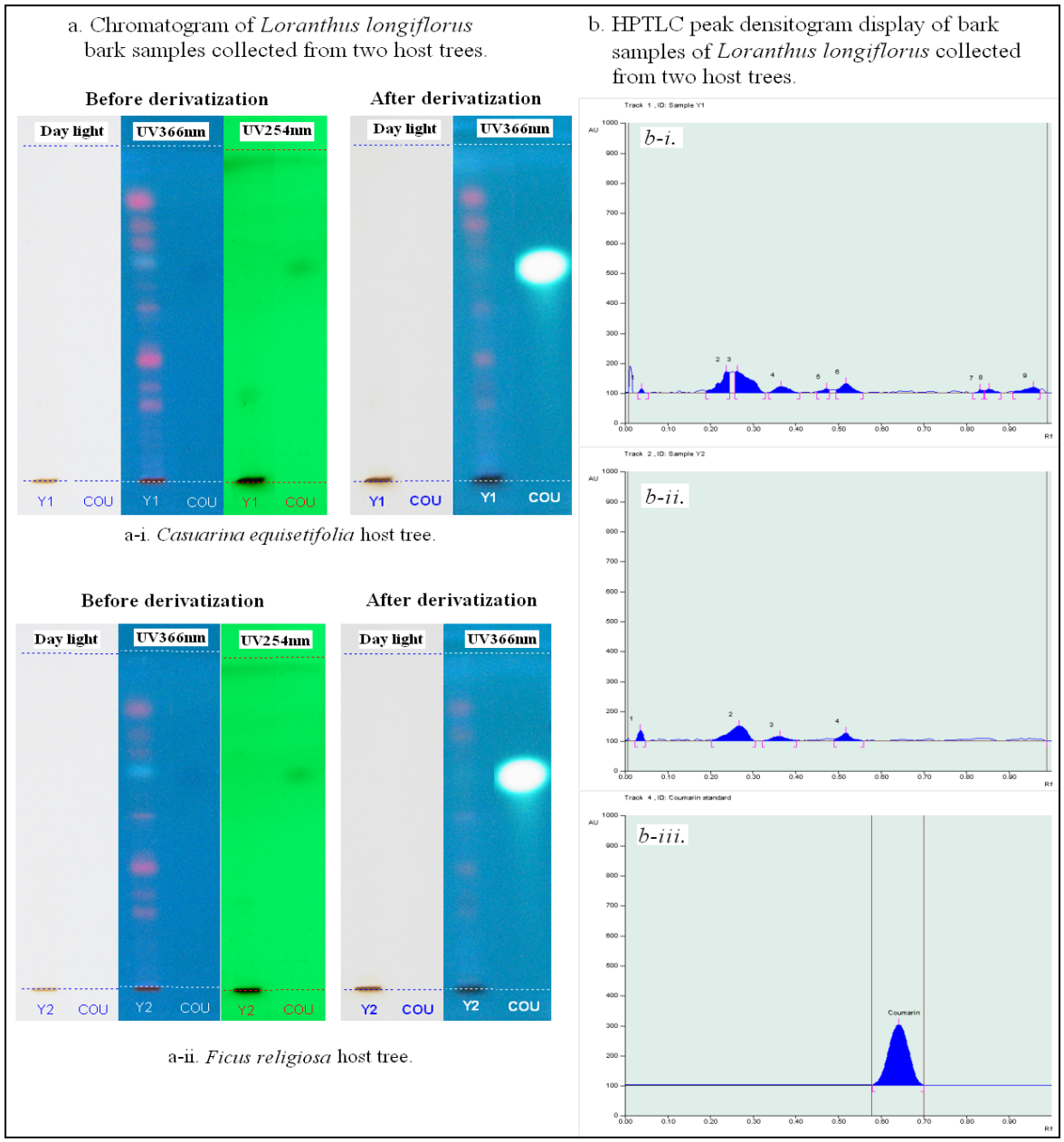

Fig 3: Chromatogram (a) and peak densitogram (b) shows coumarin profile in the *Loranthus longiflorus* bark samples collected from *Casuarina equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (Y1/Y2-sample code; COU-Coumarin standard -b-iii).

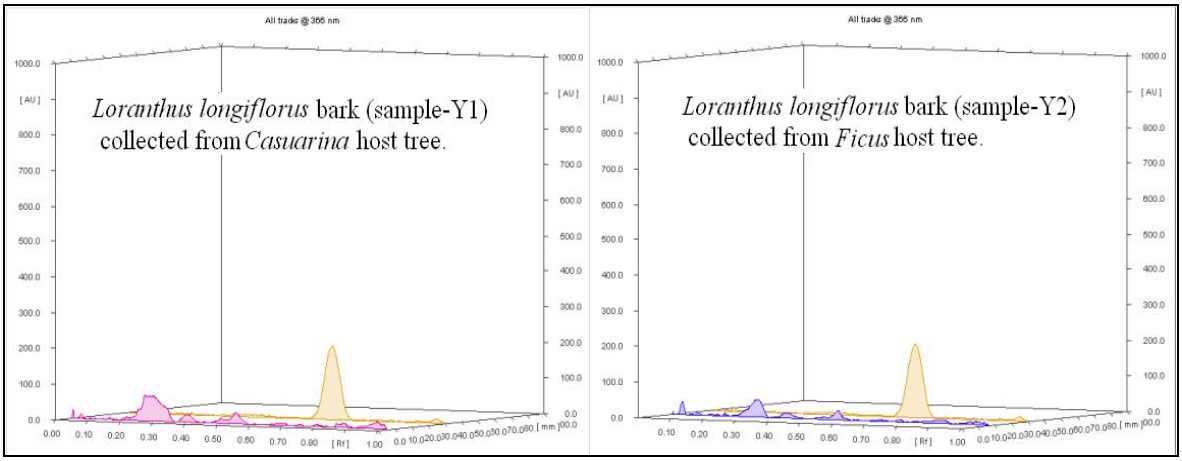

Fig 4: 3D display of densitogram showing all tracks –*Loranthus longiflorus* bark samples (Y1/ Y2) and standard (Coumarin-Orange coloured) scanned at 366nm.

Table 1: Peak table for HPTLC analysis of coumarin profile in the methanol extract of *L. longiflorus* leaf (X1/X2) samples collected from *C. equisetifolia* (X1) and *F. religiosa* (X2) host tree.

Track sample	Peak	Rf	Height	Area	Assigned substance
X1	1	0.01	184.3	1132.4	Unknown
X1	2	0.06	47.5	947.4	Coumarin 1
X1	3	0.16	80.8	1792.2	Unknown
X1	4	0.22	36.9	1110.3	Unknown
X1	5	0.30	77.8	2770.7	Unknown
X1	6	0.43	22.0	684.5	Unknown
X1	7	0.51	22.2	541.6	Unknown
X1	8	0.55	22.7	703.0	Unknown
X1	9	0.70	13.4	348.1	Coumarin 2
X1	10	0.94	40.8	1777.0	Coumarin 3
X2	1	0.22	26.4	688.8	Unknown
X2	2	0.29	46.0	1235.5	Unknown
X2	3	0.35	29.0	708.1	Unknown
X2	4	0.40	50.6	2902.4	Unknown
X2	5	0.49	32.3	1465.2	Unknown
X2	6	0.62	40.4	1185.0	Unknown
X2	7	0.67	26.7	857.3	Unknown
X2	8	0.73	12.8	184.9	Coumarin 1
X2	9	0.94	12.2	339.7	Coumarin 2
Control	1	0.64	322.7	18563.4	Coumarin standard

Table 2: Peak table for HPTLC analysis of coumarin profile in the methanol extract of *L. longiflorus* bark (Y1/Y2) samples collected from *C. equisetifolia* (Y1) and *F. religiosa* (Y2) host tree.

Track sample	Peak	Rf	Height	Area	Assigned substance
Y1	1	0.04	13.4	101.4	Unknown
Y1	2	0.24	72.2	1542.3	Unknown
Y1	3	0.26	72.0	2420.8	Unknown
Y1	4	0.36	21.3	631.1	Unknown
Y1	5	0.47	14.1	221.6	Unknown
Y1	6	0.52	31.5	813.9	Unknown
Y1	7	0.83	10.4	104.5	Unknown
Y1	8	0.85	12.0	226.8	Unknown
Y1	9	0.96	19.0	583.2	Unknown
Y2	1	0.04	35.3	365.7	Unknown
Y2	2	0.27	49.4	1997.2	Unknown
Y2	3	0.36	14.8	477.4	Unknown
Y2	4	0.52	26.6	622.6	Unknown
Control	1	0.64	204.7	9015.4	Coumarin standard

The leaf (X) and bark (Y) samples of *L. longiflorus* from *C. equisetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees shows no similar peak R_f values in the compounds detected. However, one coumarin compound (peak no. 10 of X1 and peak no. 9 of X2) and one unknown compound (peak no. 4 of X1 and peak no. 1 of X2) of *L. longiflorus* leaf samples collected from *C. equisetifolia* and *F. religiosa* host trees showing same peak R_f values (0.94 & 0.22, respectively). On the other hand, the bark samples (Y1 & Y2) of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees show three identical unknown compounds (peak no. 1,4 & 6 of Y1 and 1, 3 & 4 of Y2) with similar peak R_f values (0.04, 0.36 & 0.52).

The HPTLC analysis of methanol extracts of *L. longiflorus* leaf and bark samples from *C. equisetifolia* and *F. religiosa* host trees make certain the presence of coumarin and the host trees showed impact on the nature and number of coumarin present in the hemiparasitic plants. This study confirms the use of these plants in traditional medicine and the phytochemical analysis data will be helpful for the standardization and quality control of precious indigenous drug and also pharmaceutical industries

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