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IMPACT OF HOST TREES ON STEROID COMPOUNDS PROFILE IN THE LEAF AND BARK SAMPLES OF A HEMIPARASITE -*LORANTHUS LONGIFLORUS* DESR ANALYZED BY HPTLC.

L. Chandrakasan and R. Neelamegam*

PG Department of Botany and Research Centre S.T. Hindu College, Nagercoil -629 002, Kanyakumari (Dist.), Tamil Nadu, India.

***Correspondence for Author: Dr. R. Neelamegam**
PG Department of Botany and Research Centre S.T. Hindu College, Nagercoil -629 002, Kanyakumari (Dist.), Tamil Nadu, India.

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ABSTRACT

Influence of host plants on the steroids profile of *Loranthus longiflorus* leaf and bark samples collected from *Casuarina equisetifolia* and *Ficus religiosa* host trees were determined by HPTLC method. The methanol extract of *L. longiflorus* leaf samples obtained from *C. equisetifolia* and *F. religiosa* host trees showed 9 and 7 compounds, respectively, and were compared with stigmasterol standard. Among the compounds, 4 compounds in each sample, was identified as steroids while other compounds were unknown. Three compounds from each *L. longiflorus* leaf samples collected from *C. equisetifolia* (peak no. 6, 7 & 9) and *F. religiosa* (peak no. 4, 5 & 7) host trees showed similar R_f values (0.68, 0.80 & 0.89, respectively). On the other hand, the methanol extract of *L. longiflorus* bark sample collected from *C. equisetifolia* and *F. religiosa* host trees contained 10 and 6 compounds in each sample, respectively and were compared with solasodine standard. Among the compounds, 1 compound was identified as glycosides in the bark sample of *L. longiflorus* collected from *C. equisetifolia* while all other compounds were unknown and 2 compounds from both bark samples obtained from *C. equisetifolia* (peak no. 1 & 10) and *F. religiosa* (peak no. 1 & 6) showing similar R_f values (0.02 & 0.97). One compound (peak no. 1) of *L. longiflorus* leaf/bark samples, from *C. equisetifolia* showing similar R_f value (0.02) while none of the compounds from leaf and park samples of *L. longiflorus* collected from *F. religiosa* showing similar R_f values. The results of present study indicate that 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 steroid compounds and the host trees influenced on the nature and number of steroids present in the hemiparasitic plants.

KEYWORDS: HPTLC analysis, Steroids, Hemiparasite, *Loranthus longiflorus*, Leaf sample, Bark sample, Methanol extracts.

INTRODUCTION

Medicinal herbs have been use in one form or another under indigenous systems of medicine. Dubey[1] mentioned that the complete phytochemical investigations of medicinal plants of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers.[2-4] Plants have had a major impact on modern medicine in providing important steroids. Many types of plant steroids exist and play important roles in the biological processes of plants, such as growth and development, cell division, and resistance to damage from environmental stresses like cold weather. There are many steroids and sterols that are important in health and medicine, and some that may be used as medications. *Loranthus* species, in semiparasitic plants, are known to produce a variety of bioactive compounds. *Loranthus longiflorus* (Syn.: *Loranthus falcate*/*Dendrophthoe falcata*) possesses remarkable potentials as a medicinal plant evident from the wound healing, anti-microbial, anti-oxidant, antinociceptive properties of its ethanol extracts.[5-7] Medicinal properties of this hemiparasite may vary in effects respective to different hosts it establishes a relation with.[8, 9] The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the steroid compounds profile in the leaf and bark samples of *Loranthus longiflorus*, a hemiparasite.

MATERIALS AND METHODS

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.

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.

Preparation of extract
The dried plant materials of *L. longiflorus* leaf and 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.

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 steroid compounds.

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.

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.

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.

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.

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. [10]

HPTLC analysis for steroid

- ***Test solution***: Methanol extracts of *L. longiflorus* leaf/bark samples obtained from *C. equisetifolia* and *F. religiosa* host trees.
- ***Standard solution***: Methanol.
- ***Standard chemical***: SGL – Stigmasterol was used as reference standard compound.
- ***Mobile phase***: Chloroform-Acetone (8: 2).
- ***Spray reagent***: Anisaldehyde sulphuric acid reagent.

RESULTS and DISCUSSION
HPTLC analysis has been done to analyze steroids in the leaf and bark samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees and the results are presented in Figures: 1 to 4 and Tables: 1 and 2.

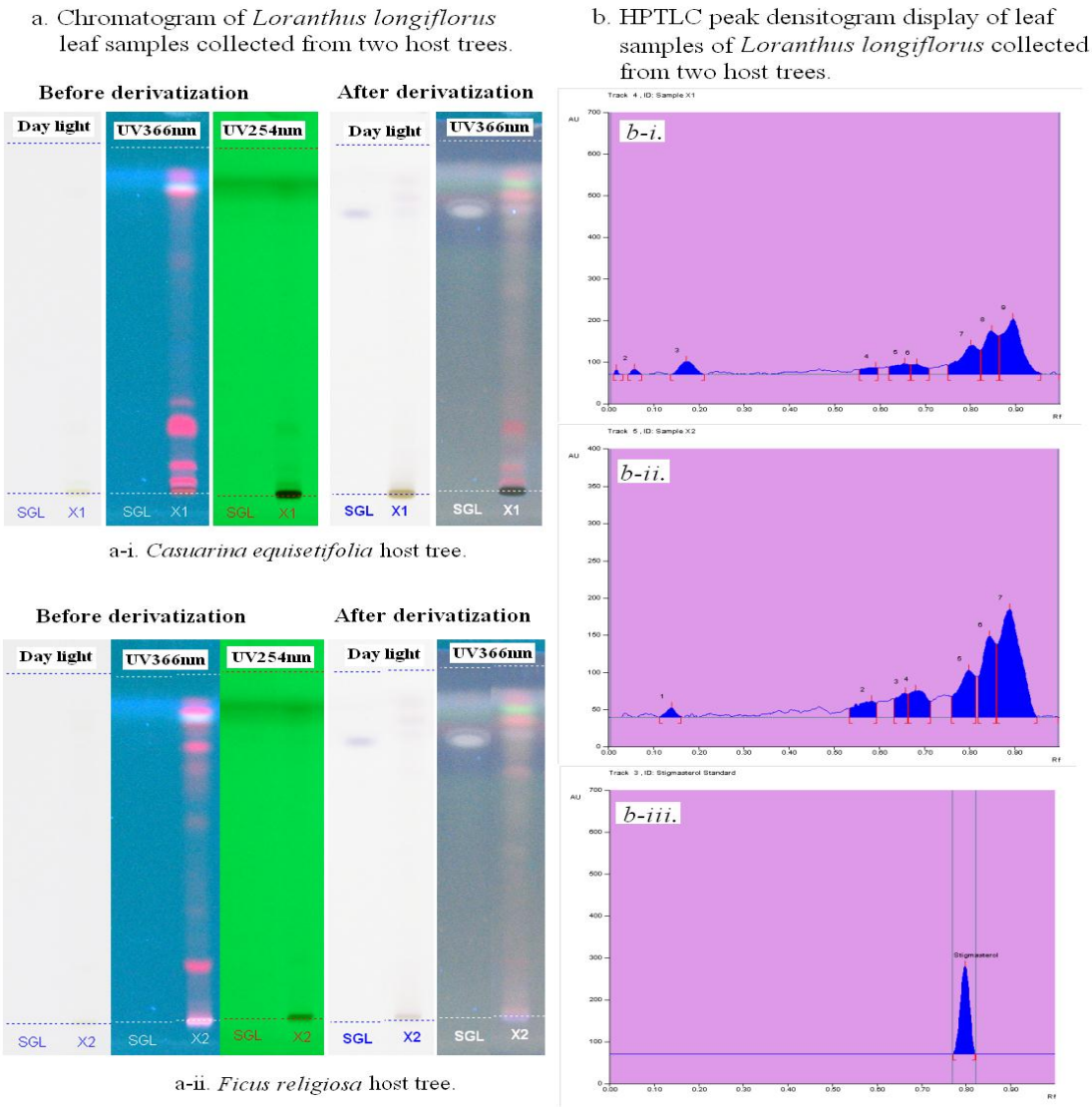

Figure 1: Chromatogram (a) and peak densitogram (b) shows steroids profile in the *Loranthus longiflorus* leaf samples collected from *Casuarina equisetifolia*(a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; SGL-Stigmasterol standard -b-iii).

The chromatogram (Figure: 1a & 3a) shows steroid profile of methanol extract of *L. longiflorus* leaf (X1/X2) and bark (Y1/Y2) samples collected from *C. equisetifolia* (Figure: 1a-i & 2a-i) and *F. religiosa* (Figure: 1a-ii & 2a-ii) host trees and is compared with stigmasterol (SGL -for leaf samples-X) and solasodine (SOE -for bark samples-Y) standards. Blue, blue-violet coloured zones at day light and UV 366nm mode present in the stigmasterol/solasodine standards and plant samples tracks were observed in the chromatogram after derivatization and this confirmed the presence of steroid compounds in the leaf and bark samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees.

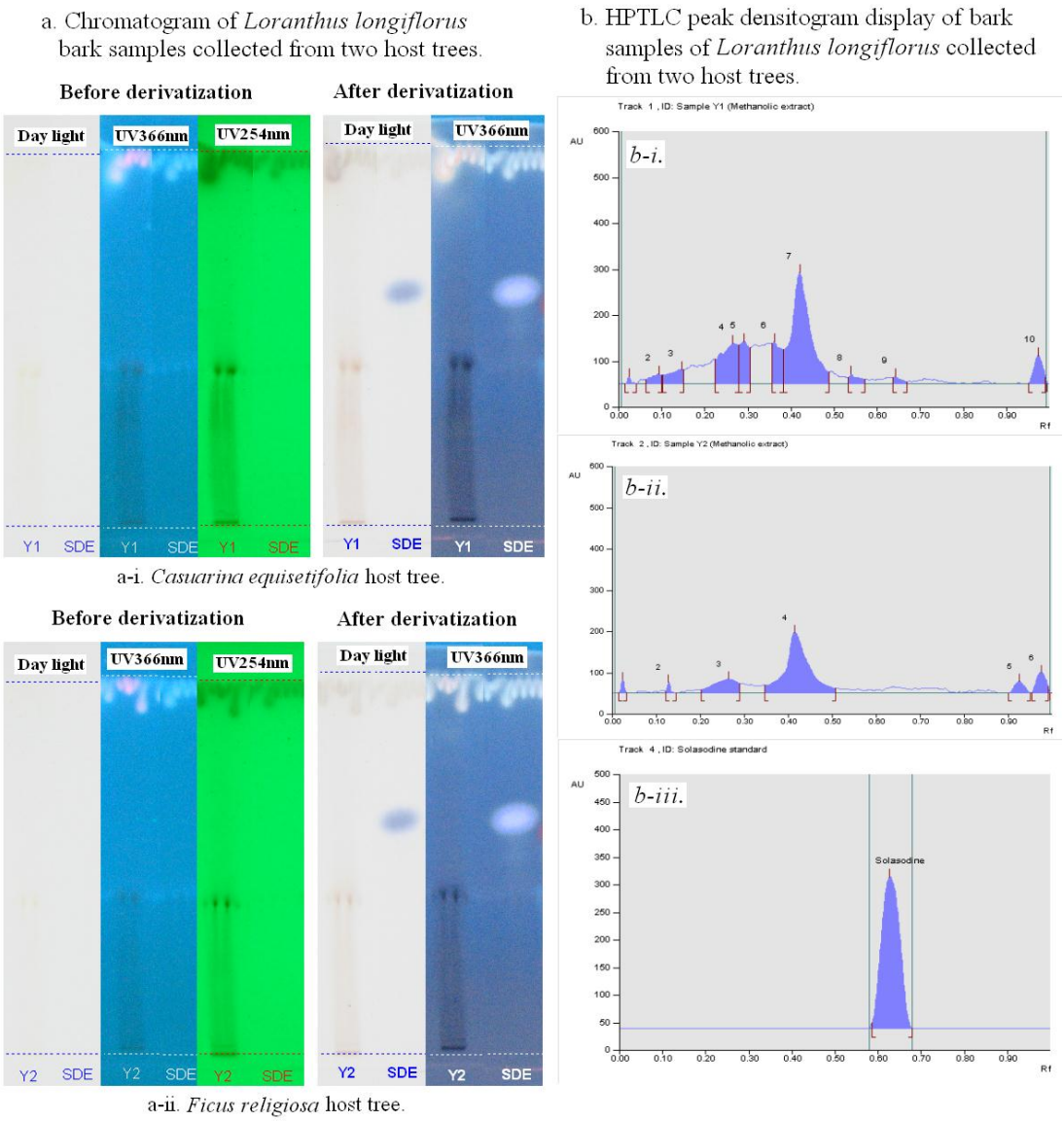

Figure 2: Chromatogram (a) and peak densitogram (b) shows steroids profile in the *Loranthus longiflorus* bark samples collected from *Casuarina equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (Y1/Y2-sample code; SOE- Solasodine standard -b-iii).

The densitogram (Figure: 1b & 2b) shows the profile of steroid compounds present in the methanol extract of *L. longiflorus* leaf (Figure: 1b) and bark (Figure: 2b) samples collected from *C. equisetifolia* (Figure: 1b-i/2b-i) and *F. religiosa* (Figure: 1b-ii/2b-ii) host trees; and stigmasterol standard for leaf (Figure: 1b-iii) and solasodine standard for bark (Figure: 2b-iii) samples scanned at 366nm and 500nm, respectively.

Densitogram-3D display for glycoside profile shows all tracks of *L. longiflorus* leaf (X1/X2) and bark (Y1/Y2) samples collected from *C. equisetifolia* (X1&Y1) and *F. religiosa* (X2/Y2) host trees and stigmasterol and solasodine standards scanned at 366nm and 500nm, respectively (Figure: 3 & 4).

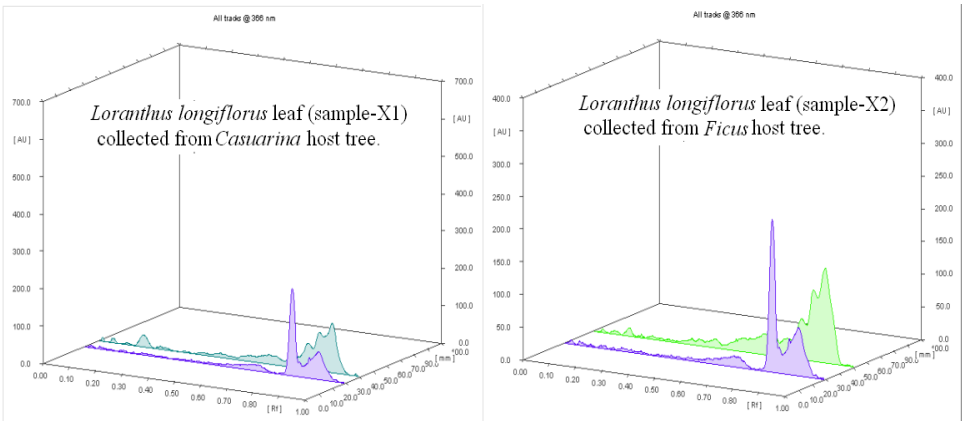

Figure 3: Densitogram-3D display showing all tracks –*Loranthus longiflorus* leaf samples (X1/X2) and standard (Stigmasterol -blue coloured) scanned at 366nm.

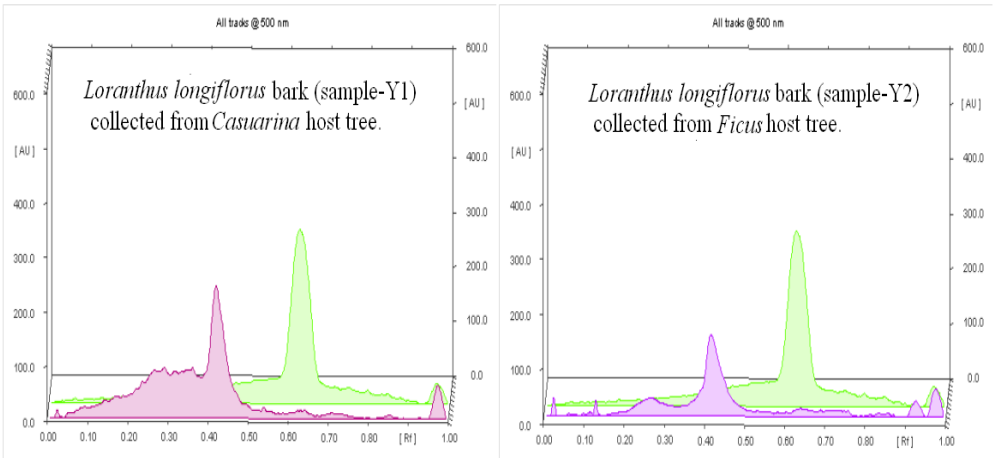

Figure 4: Densitogram-3D display showing all tracks –*Loranthus longiflorus* bark samples (Y1/Y2) and standard (Solasodine -Green coloured) scanned at 500nm.

HPTLC analysis of *Loranthus longiflorus* leaf sample

HPTLC analysis for steroid profile in the methanol extract of *L. longiflorus* leaf (X) samples collected from *C. equisetifolia* (X1) and *F. religiosa* (X2) host trees showed several peaks (R_f -values) of compounds (Table: 1-X1/X2; Figure: 1b-i/b-ii) and were compared with stigmasterol (SGL) and solasodine (SOE) standards.

Table 1: Peak table for HPTLC analysis of steroids profile in the methanol extract of *Loranthus longiflorus* leaf (X1/X2) samples collected from *Casuarina equisetifolia* (X1) and *Ficus religiosa* (X2) host tree.

Track sample	Peak	Rf	Height	Area	Assigned substance
X1	**1**	**0.02**	**10.7**	**67.8**	**Unknown**
X1	2	0.06	12.0	165.1	Unknown
X1	3	0.17	31.1	933.0	Unknown
X1	4	0.59	16.5	504.7	Unknown
X1	5	0.65	25.8	889.0	Unknown
X1	**6**	**0.68**	**24.7**	**665.5**	**Steroid 1**
X1	**7**	**0.80**	**69.5**	**2763.2**	**Steroid 2**
X1	8	0.85	104.3	2932.3	Steroid 3
X1	**9**	**0.89**	**132.9**	**5134.9**	**Steroid 4**
X2	1	0.14	12.6	246.1	Unknown
X2	2	0.58	21.5	877.2	Unknown
X2	3	0.66	32.1	727.1	Unknown
X2	**4**	**0.68**	**35.8**	**1290.4**	**Steroid 1**
X2	**5**	**0.80**	**63.1**	**2119.4**	**Steroid 2**
X2	6	0.84	108.8	3040.5	Steroid 3
X2	**7**	**0.89**	**145.3**	**6168.4**	**Steroid 4**
Control	**1**	**0.80**	**232.4**	**4871.6**	**Stigmasterol standard**

The methanol extract of *L. longiflorus* leaf samples (X1) obtained from *C. equisetifolia* host trees showed 9 compounds (Table: 1X-1; Figure: 1b-i) with peak R_f values ranging from 0.02 to 0.89, peak height ranging from 10.7 to 132.9 and peak area ranging from 67.8 to 5134.9 as compared to stigmasterol (Figure: 1b-iii) standard (0.80, 232.4 and 4871.6, respectively). Among the 9 compounds detected, 3 were identified as steroids (peak no. 6, 7 & 9) and others were unknown.

On the other hand, the methanol extract of *L. longiflorus* leaf (X2) sample collected from *F. religiosa* host tree showed 7 compounds (Table: 1X-2; Figure: 1b-ii) with peak R_f values ranging from (0.14 to 0.89, peak height from 12.6 to 145.3 and peak area from 246.1 to 6168.4 as compared to stigmasterol (Figure: 1b-iii) standard (0.80, 232.4 and 4871.6, respectively) and out of 7 compounds detected, 4 were identified as steroids (peak no. 4-7) and others were unknown.

HPTLC analysis of *Loranthus longiflorus* bark sample

HPTLC analysis for steroid profile in the methanol extract of *L. longiflorus* bark (Y) samples collected from *C. equisetifolia* (Y1) and *F. religiosa* (Y2) host trees showed several peaks (R_f -values) of compounds (Table: 2Y2/Y2) and were compared with stigmasterol (SGL) and solasodine (SOE) standards.

Table 2: Peak table for HPTLC analysis of steroids profile in the methanol extract of *Loranthus longiflorus* bark (Y1/Y2) samples collected from *Casuarina equisetifolia* (Y1) and *Ficus religiosa* (Y2) host tree.

Track sample	Peak	Rf	Height	Area	Assigned substance
Y1	**1**	**0.02**	**14.6**	**96.4**	**Unknown**
Y1	2	0.09	20.4	487.5	Unknown
Y1	3	0.15	31.4	1016.1	Unknown
Y1	4	0.27	87.2	3216.8	Unknown
Y1	5	0.29	92.0	1886.1	Unknown
Y1	6	0.36	91.4	1798.8	Unknown
Y1	7	0.42	241.5	9646.4	Unknown
Y1	8	0.54	20.2	414.9	Steroid 1
Y1	9	0.64	15.6	269.0	Unknown
Y1	**10**	**0.97**	**61.7**	**1145.9**	**Unknown**
Y2	**1**	**0.02**	**33.1**	**213.6**	**Unknown**
Y2	2	0.13	27.5	172.4	Unknown
Y2	3	0.26	33.6	1683.9	Unknown
Y2	4	0.41	147.2	7079.9	Unknown
Y2	5	0.93	28.9	581.6	Unknown
Y2	**6**	**0.97**	**50.5**	**992.3**	**Unknown**
Control	**1**	**0.63**	**318.8**	**15382.6**	**Solasodine standard**

The methanol extract of *L. longiflorus* bark samples (Y1) collected from *C. equisetifolia* host tree showed 10 compounds (Table: 2Y-1; Figure: 2b-i) with varied peak R_f values (0.02-0.97), peak height (14.6-241.5) and peak area (96.4-9646.4) as compared to solasodine (Figure: 2b-iii) standard (0.63, 318.8 and 15382.6, respectively). Out of 10 compounds detected, one compound (peak no. 8) was identified as steroid and others were unknown.

Similarly, the methanol extract of *L. longiflorus* bark (X2) sample collected from *F. religiosa* host tree revealed 6 compounds (Table: 2Y-2; Figure: 2b-ii) with peak R_f values ranging from 0.02 to 0.97, peak height from 27.5 to 147.2 and peak area from 172.4 to 7079.9 as compared to solasodine (Figure: 2b-iii) standard (0.63, 318.8 and 1538.6, respectively). All the 6 compounds detected were unknown.

The leaf (X1) and bark (Y1) samples of *L. longiflorus* from *C. equisetifolia* host trees showed one unknown compound (peak no. 1 of X1 & Y1) with same peak R_f values (0.02) in the compounds detected (Table: 1 & 2). The leaf (X2) and bark (Y2) samples of *L. longiflorus* from *F. religiosa* host tree showed no similarities in their peak R_f values of compounds detected (Table: 1 & 2).

In general, three steroid compounds (peak no. 6, 7 & 9 of X1 and peak no. 4, 5 & 7 of X2) of *L. longiflorus* leaf samples from *C. equisetifolia* and *F. religiosa* host trees shows same peak R_f values (0.68, 0.80 & 0.89), respectively (Table: 1), while the *L. longiflorus* bark samples (Y1 & Y2) shows two identical steroid compounds (peak no. 1 & 10 of Y1 and 1 & 6 of Y2) with similar peak R_f values (0.02 & 0.97), respectively (Table: 2).

The results of present study indicate that 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 steroid compounds and the host trees influenced on the nature and number of steroids present in the hemiparasitic plants.

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