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HPTLC DETERMINATION OF TERPENOID PROFILE IN THE LEAF AND BARK SAMPLES OF *LORANTHUS LONGIFLORUS* DESR COLLECTED FROM TWO HOST TREES

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ABSTRACT

HPTLC determination of terpenoid compounds profile was carried out in the leaf and bark samples of *Loranthus longiflorus* collected from two host trees –*Casuarina equisetifolia* and *Ficus religiosa*. The methanol extract of *L. longiflorus* leaf samples obtained from *C. equisetifolia* and *F. religiosa* host trees showing 10 compounds in each samples and were compared with solanesol standard. Among the compounds, all (10) and 9 compounds, in each sample, respectively, was identified as terpenoids while other compounds were unknown. Three compounds from each *L. longiflorus* leaf samples collected from *C. equisetifolia* (peak no. 1, 6 & 7) and *F. religiosa* (peak no. 2, 7 & 8) host trees showed similar R_f values (0.14, 0.47 & 0.56, respectively). On the other hand, the methanol extract of *L. longiflorus* bark sample collected from *C. equisetifolia* and *F. religiosa* host trees contained 8 and 6 compounds in each sample, respectively, and were compared with solanesol standard. Among the compounds, 4 and 5 compounds were identified as terpenoids in the bark sample of *L. longiflorus* collected from *C. equisetifolia* and F. religiosa, respectively, while other compounds were unknown and 3 compounds from both bark samples obtained from C. equisetifolia (peak no. 2, 7 & 8) and *F. religiosa* (peak no. 1, 5 & 6) showing similar R_f values (0.04, 0.76 & 0.92). One compound of *L. longiflorus* leaf (peak no. 1) /bark (peak no. 3) samples, from *C. equisetifolia* showing similar R_f value (0.14) and one compound in leaf (peak no. 7) and park (peak no. 2) samples of *L. longiflorus* collected from *F. religiosa* showing similar R_f value (0.47). The results 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 many terpenoid compounds and the host trees influenced on the nature and number of terpenoids present in the hemiparasitic plants.

1. INTRODUCTION

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of plants for secondary metabolites. The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, antianalgesic and antidiuretic can be attributed to their high steroids, tannins, terpenoids and saponins. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification. Terpenoids (also called "isoprenoids") constitute one of the largest families of natural products. It accounts for more than 40,000 individual compounds of both primary and secondary metabolisms. Most of them are of plant origin, and hundreds of new structures are reported every year[1-3]. All organisms naturally produce some terpenoids as part of primary metabolism, but many produce terpenoids via secondary metabolism. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. The terpenoids have been reported to protect lipid, blood and body fluids against the attack of reactive oxygen species (free radicals and prevent the occurrence of cancer in many tissues of human body[4-7]. They are also known to have antimicrobial and bactericidal properties against several pathogenic bacteria[8-12].

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[13-15]. Medicinal properties of this hemiparasite may vary in effects respective to different hosts it establishes a relation with[16, 17]. The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the terpenoid compound profile in the leaf and bark samples of *Loranthus longiflorus* Desr, 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 terpenoid compounds.

2.4.1. 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.

2.4.2. 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.

2.4.3. 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.

2.4.4. 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.

2.4.5. 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[18].

2.4.6. HPTLC analysis for terpenoids

- ***Test solution***: Methanol extracts of *L. longiflorus* leaf/bark samples obtained from *C. equisetifolia* and *F. religiosa* host trees.

- ***Standard solution***: Methanol.
- ***Standard chemical***: SOL – Solanesol was used as reference standard compound.
- ***Mobile phase***: n-Hexane-Ethyl acetate (7.2: 2.9).
- ***Spray reagent***: Anisaldehyde sulphuric acid reagent.

3. RESULTS AND DISCUSSION

HPTLC analysis for terpenoid profile in the methanol extract of *L. longiflorus* leaf and bark samples collected from two host trees was carried out and the results are presented in Table 1 and 2; Figure 1 to 4.

The chromatogram (Figure 1a & 2a) shows terpenoid profile of methanol leaf (X) and bark (Y) extract of *Loranthus* samples collected from *C. equisetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees and is compared with solanesol (SOL) standard. Blue-violet coloured zones at day light mode present in the solanesol standard and plant samples track at UV 500nm mode were observed in the chromatogram after derivatization and this confirmed the presence of terpenoid compounds in the leaf and bark samples of *L. longiflorus*.

The densitogram (Figure 1b/2b) shows the profile of terpenoid compounds present in the methanol extract of *L. longiflorus* leaf (X) and bark (Y) samples collected from *C. equisetifolia* (Figure 1b-i and 2b-i) and *F. religiosa* (Figure 2b-i and 2b-ii) host trees; and solanesol standard for leaf (Figure 1b-iii) and bark (Fig.-3b-iii) samples scanned at UV 500nm.

The 3D display of densitogram for terpenoid profile 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 solanesol (SOL) standard scanned at 500nm (Figure 3 and 4).

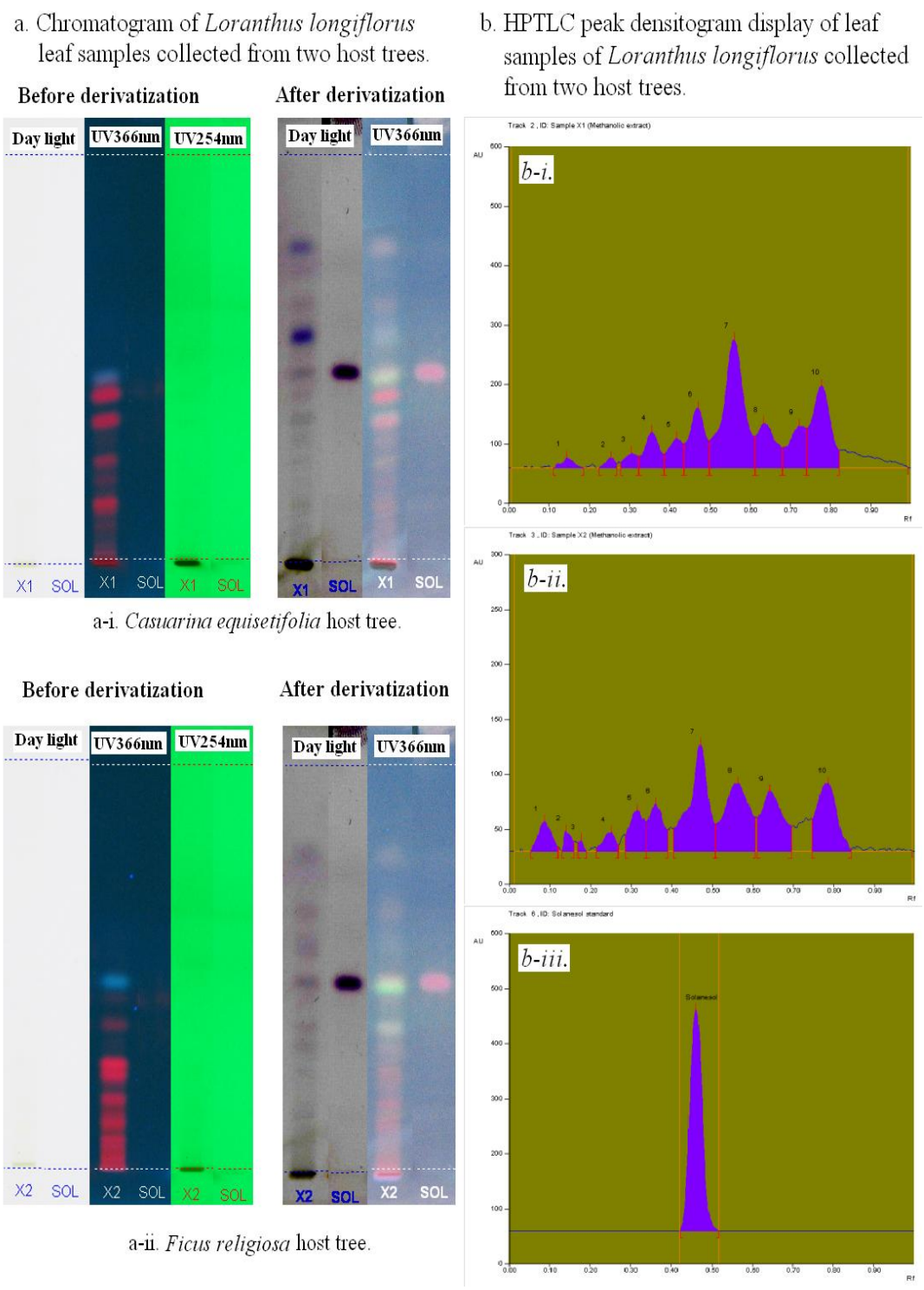

Figure 1: Chromatogram (a) and peak densitogram (b) shows terpenoids profile in the *Loranthus longiflorus* leaf samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; SOL-Solanesol standard -b-iii).

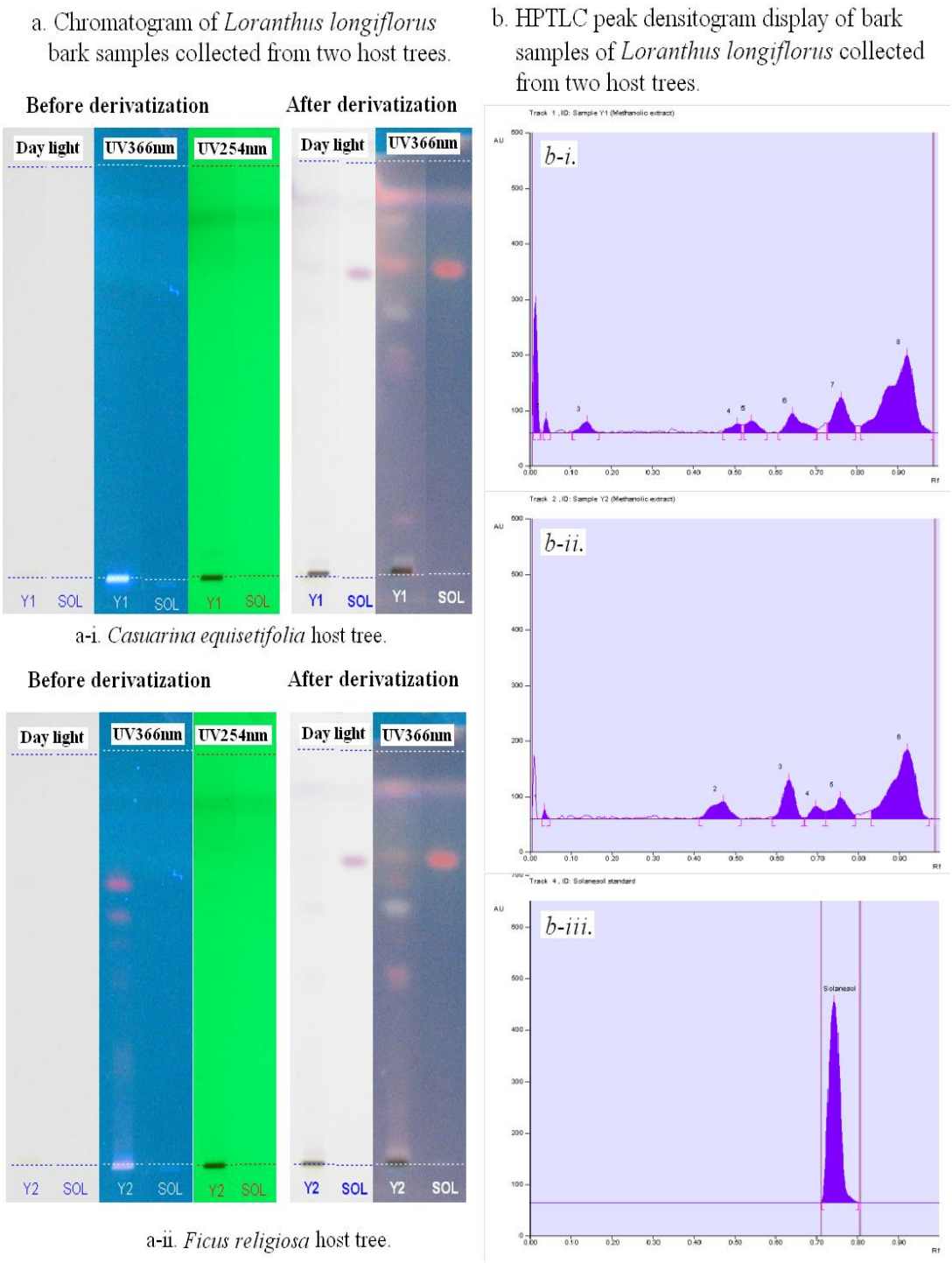

Figure 2: Chromatogram (a) and peak densitogram (b) shows terpenoids profile in the *Loranthus longiflorus* bark samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (Y1/Y2-sample code; SOL-Solanesol standard -b-iii).

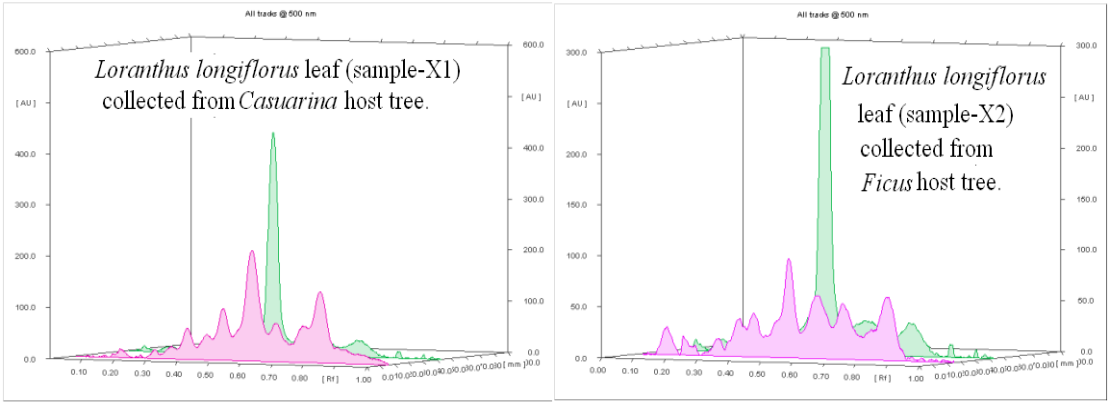

Figure 3: 3D display of densitogram showing all tracks –Loranthus longiflorus leaf samples (X1/ X2) and standard (Solanesol-green coloured) scanned at 500nm.

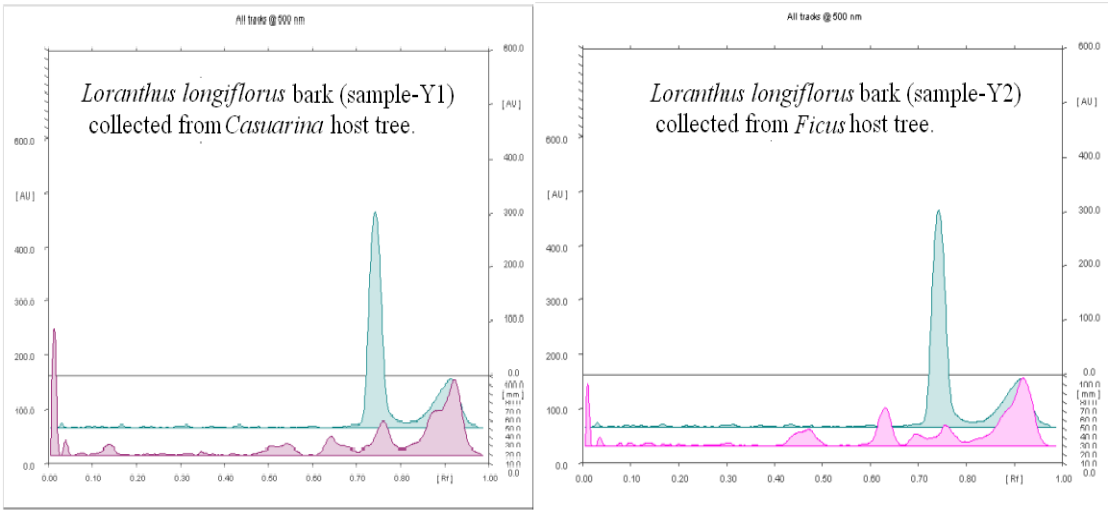

Figure 4: 3D display of densitogram showing all tracks –*Loranthus longiflorus* bark samples (Y1/ Y2) and standard (Solanesol-green coloured) scanned at 500nm.

HPTLC analysis for terpenoid profile in the methanol extract of *L. longiflorus* leaf samples collected from *C. equisetifolia* (X1) and *F. religiosa* (X2) host trees showed several peaks (R_f -values) of compounds (Table 1; Figure 1) and were compared with solanesol (SOL) standard.

The methanol extract of *L. longiflorus* leaf samples (X1) obtained from *C. equisetifolia* host trees showed 10 compounds (Table 1X-1; Figure 1b-i) with peak R_f values ranging from 0.14 to 0.78, peak height ranging from 17.0 to 215.6 and peak area ranging from 360.1 to 10321.7 as compared to solanesol standard (0.46, 434.3 and 14285.0, respectively). All the 10 compounds detected were identified as terpenoids.

Table 1: Peak table for HPTLC analysis of terpenoids 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**	**0.14**	**17.0**	**454.3**	**Terpenoid 1**
X1	2	0.26	16.4	360.1	Terpenoid 2
X1	3	0.30	24.4	700.2	Terpenoid 3
X1	4	0.35	60.7	1963.7	Terpenoid 4
X1	5	0.42	49.8	1585.5	Terpenoid 5
X1	**6**	**0.47**	**101.0**	**3498.2**	**Terpenoid 6**
X1	**7**	**0.56**	**215.6**	**10321.7**	**Terpenoid 7**
X1	8	0.63	74.5	3089.2	Terpenoid 8
X1	9	0.72	70.8	2641.4	Terpenoid 9
X1	10	0.78	138.1	5887.1	Terpenoid 10
X2	1	0.09	27.6	831.7	Terpenoid 1
X2	**2**	**0.14**	**18.7**	**300.6**	**Terpenoid 2**
X2	3	0.18	10.3	117.6	Unknown
X2	4	0.25	17.4	488.1	Terpenoid 3
X2	5	0.32	37.3	1231.9	Terpenoid 4
X2	6	0.36	43.4	1400.7	Terpenoid 5
X2	**7**	**0.47**	**97.5**	**4146.3**	**Terpenoid 6**
X2	**8**	**0.56**	**61.9**	**3539.6**	**Terpenoid 7**
X2	9	0.64	54.9	2672.3	Terpenoid 8
X2	10	0.79	62.1	2875.2	Terpenoid 9
Control	**1**	**0.46**	**434.3**	**14285.0**	**Solanesol standard**

On the other hand, the methanol extract of *L. longiflorus* leaf sample collected from *Ficus* host tree showed 10 compounds (Table 1X-2; Figure 3b-ii) with peak R_f values ranging from (0.09 to 0.79, peak height from 10.3 to 97.5 and peak area from 117.6 to 4146.3 as compared to solanesol standard (0.46, 434.3 and 14285.0, respectively) and out of 10 compounds, 9 were identified as terpenoids and the remaining one was unknown.

HPTLC analysis for terpenoid profile in the methanol extract of *L. longiflorus* bark samples collected from *C. equisetifolia* (Y1) and *F. religiosa* (Y2) host trees showed several peaks (R_f -values) of compounds (Table 2) and were compared with solanesol (SOL) standard.

The methanol extract of *L. longiflorus* bark samples (Y1) collected from *C. equisetifolia* host tree showed 8 compounds (Table 2Y-1; Figure 2b-i) with varied peak R_f values (0.01-0.92), peak height (16.3-235.2) and peak area (232.5-8127.8) as compared to solanesol standard (0.74, 400.9 and 10819.7, respectively). Out of 8 compounds detected, 5 compounds (peak no. 2, 3, 6-8) were identified as terpenoids and others were unknown.

Similarly, the methanol extract of *L. longiflorus* bark (Y) sample collected from *F. religiosa* host tree revealed 6 compounds (Table 2Y-2; Figure 3b-ii) with peak R_f values ranging from 0.04 to 0.92, peak height from 16.4 to 124.7 and peak area from 128.7 to 6362.5 as compared to standard solanesol (0.74, 400.9 and 10819.7, respectively). Among the 6 compounds detected, 5 were identified as terpenoids (peak no. 1-3, 4 & 5) and the other one was unknown.

Table 2: Peak table for HPTLC analysis of terpenoids 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	0.01	235.2	2073.6	Unknown
Y1	**2**	**0.04**	**26.6**	**232.5**	**Terpenoid 1**
Y1	**3**	**0.14**	**20.0**	**507.2**	**Terpenoid 2**
Y1	4	0.51	16.3	441.1	Unknown
Y1	5	0.54	21.3	631.1	Unknown
Y1	6	0.64	35.3	1318.1	Terpenoid 3
Y1	**7**	**0.76**	**63.9**	**2083.9**	**Terpenoid 4**
Y1	**8**	**0.92**	**140.0**	**8127.8**	**Terpenoid 5**
Y2	**1**	**0.04**	**16.4**	**128.7**	**Terpenoid 1**
Y2	**2**	**0.47**	**31.6**	**1352.2**	**Terpenoid 2**
Y2	3	0.63	70.4	1895.7	Terpenoid 3
Y2	4	0.70	23.0	595.9	Unknown
Y2	**5**	**0.76**	**39.0**	**1292.7**	**Terpenoid 4**
Y2	**6**	**0.92**	**124.7**	**6362.5**	**Terpenoid 5**
Control	**1**	**0.74**	**400.9**	**10819.7**	**Solanesol standard**

The leaf (X) and bark (Y) samples of *L. longiflorus* from *C. equisetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees showed each one compound (peak no. 1 & 3 of X1/Y1 and peak no. 7 & 2 of X2/Y2, respectively) similar with same peak R_f value (0.14 & 0.47, respectively) in the compounds detected. In general, the three terpenoid compounds (peak no. 1, 6 & 7 of X1 and peak no. 1, 7, & 8 of X2) of the leaf samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees showed (Tab.-1) same peak R_f values (0.14, 0.47 & 0.56, respectively). On the other hand, the bark samples (Y1 & Y2) of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees showed (Tab.-2) three identical terpenoid compounds (peak no. 2, 7 & 8 of Y1 and 1, 5 & 6 of Y2) with similar peak R_f values (0.04, 0.76 & 0.92).

4. CONCLUSION

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 terpenoid compounds and the host trees influenced on the nature and number of terpenoids present in the hemiparasitic plants.

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