DETERMINATION OF PHENYL PROPANOIDS PROFILE IN THE LEAF AND BARK SAMPLES OF *LORANTHUS LONGIFLORUS* DESR COLLECTED FROM TWO HOST TREES BY HPTLC.

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ABSTRACT

Influence of host plants on the phenyl propanoids 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 6 and 7 compounds, respectively and were compared with eugenol standard. Among the compounds, 5 and 6 compounds in each sample, respectively, was identified as phenyl propanoids while the remaining one unknown. Two compounds from each *L. longiflorus* leaf samples collected from *C. equisetifolia* (peak no. 2 & 3) and *F. religiosa* (peak no. 2 & 3) host trees showed similar *R*<sub>f</sub> values (0.16 & 0.27, 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 eugenol standard. Among the compounds, 5 and 3 compounds, in each sample, was identified as phenyl propanoids while other compounds were unknown and 2 compounds from both bark samples obtained from *C. equisetifolia* (peak no. 2 & 6) and *F. religiosa* (peak no. 1 & 4) showed similar *R*<sub>f</sub> values (0.21 & 0.62). None of the compounds from leaf/bark samples of *L. longiflorus* collected from *C. equisetifolia* showing similar *R*<sub>f</sub> values while one compound (peak no. 7 & 6) of *L. longiflorus* leaf/bark samples, respectively, from *F. religiosa* showing similar *R*<sub>f</sub> value (0.94). 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 phenyl propanoid compounds and the host...
trees influenced on the nature and number of phenyl propanoids present in the hemiparasitic plants.

**KEYWORDS:** Hemiparasite, *Loranthus longiflorus*, Leaf/bark methanol extracts, HPTLC analysis, Phenyl propanoids, *Casuarina equisetifolia* host, *Ficus religiosa* host.

**INTRODUCTION**

Herbal medicine is still the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body.

Last few years, much interest has been attracted to natural and synthetic phenyl propanoids for various medicinal uses as antioxidant, UV screens, anticancer, wound healing, antivirus, anti-inflammatory and antibacterial agents. Phenyl propanoids belong to the largest group of secondary metabolites produced by plants, mainly, in response to biotic or abiotic stresses such as infections, wounding, UV irradiation, pollutants, and other hostile environmental conditions. It is thought that the molecular basis for the protective action of phenyl propanoids in plants is their antioxidant and free radical scavenging properties. Phenyl propanoids are parent molecules for biosynthesis of numerous structurally and functionally diverse plant polyphenols (simple phenolic acids and esters, glycosylated derivatives of primary Phenyl propanoids, flavonoids, isoflavonoids, stilbenes, coumarins, curcuminoids, lignans, etc.), which play multiple essential roles in plant physiology (Korkina et al., 2011).

These numerous phenolic compounds are major biologically active components of human diet, spices, aromas, wines, beer, essential oils, propolis and traditional medicine. The phenyl propanoids are biologically active compounds of medicinal plants which are perspective sources of the neurotropic, adaptogenic, immunostimulating and hepatoprotective preparations (Cheminat et al., 1988; Bauer and Wagner, 1990; Koch-Heitzmann and Schultze, 1991; Kurkin et al., 1991; Cometa et al., 1993; Wagner, 1993; Kurkin, 2002; Kurkin, 2003). Surh (2003) have proposed that phenyl propanoids can inhibit initiation of tumorigenesis or its development. Concentrations of phenyl propanoids within plants are also altered by changes in resource availability (Wikipedia; Davey et al., 2004).
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 ethanolic extracts (Pattanayak and Sunita, 2008; Chandrakasan and Neelamegam, 2011; 2012).[14-16] Medicinal properties of this hemiparasite may vary in effects respective to different hosts it establishes a relation with (Mallavadhani et al., 2006; Chandrakasan, 2012).[17,18] The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the phenyl propanoid compound 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 phenyl propanoid 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 60F254 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 (Shah et al., 2008).

HPTLC analysis for phenyl propanoid
- **Test solution**: Methanol extracts of *L. longiflorus* leaf/bark samples obtained from *C. equisetifolia* and *F. religiosa* host trees.
- **Standard solution**: Methanol.
- **Standard chemical**: EUG-Eugenol was used as reference standard compound.
- **Mobile phase**: Toluene-Ethyl acetate (93: 7).
- **Spray reagent**: Anisaldehyde sulphuric acid reagent.
RESULTS and DISCUSSION
The chromatogram (Figure 1a and 2a) shows phenyl propanoid 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 is compared with eugenol (EUG) standard. Blue, blue-violet coloured fluorescent zones present in the eugenol standard and plant samples tracks at UV 366nm mode were observed in the chromatogram after derivatization and this confirmed the presence of phenyl propanoid compounds in the leaf and bark samples of *L. longiflorus*.

![Chromatogram of Loranthus longiflorus leaf samples collected from two host trees.](image)

![HPTLC peak densitogram display of leaf samples of Loranthus longiflorus collected from two host trees.](image)

The chromatogram (Figure 1a and 2a) shows phenyl propanoid 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; EUG-Eugenol standard -b-iii).

Figure -1: Chromatogram (a) and peak densitogram (b) shows phenyl propanoid 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; EUG-Eugenol standard -b-iii).
Figure -2: Chromatogram (a) and peak densitogram (b) shows phenyl propanoid 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; EUG-Eugenol standard -b-iii).

The densitogram (Figure 1b and 2b) shows the profile of phenyl propanoid compounds present in the methanol extract of *L. longiflorus* leaf (Figure 1b) and bark (Figure 2b) samples.
collected from *C. equisetifolia* (Figure 1b-i and 2b-i) and *F. religiosa* (Figure 1b-ii and 2b-ii) host trees; and eugenol standard for leaf (Figure 1b-iii) and bark (Figure 2b-iii) samples scanned at 366nm.

The 3D display of densitogram for phenyl propanoid 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 eugenol standard scanned at 366nm and 500nm, respectively (Figure 3 and 4).

![Figure 3: HPTLC-3D display of densitogram showing all tracks -Loranthus longiflorus leaf samples (X1/X2) and standard (Eugenol-blue coloured) scanned at 366nm.](image)

![Figure 4: 3D display of densitogram showing all tracks –Loranthus longiflorus bark samples (Y1/Y2) and standard (Eugenol-orange coloured) scanned at 500nm.](image)

HPTLC analysis for phenyl propanoid 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; Figure 1b) and were compared with eugeol standard.

The methanol extract of *L. longiflorus* leaf samples (X1) obtained from *C. equisetifolia* host trees showed 6 compounds (Table 1X-1; Figure 1b-i) with peak $R_f$ values ranging from 0.08 to 0.98, peak height ranging from 11.0 to 92.8 and peak area ranging from 140.5 to 3000.6 as compared to eugenol standard (0.43, 124.3 and 4570.5, respectively). Among the 6 compounds detected, 5 were identified as phenyl propanoid compounds (peak no. 1-5) and the 6th compound was unknown.

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

<table>
<thead>
<tr>
<th>Track sample</th>
<th>Peak</th>
<th>$R_f$</th>
<th>Height</th>
<th>Area</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>1</td>
<td>0.08</td>
<td>20.7</td>
<td>539.6</td>
<td>Phenyl propanoid 1</td>
</tr>
<tr>
<td>X1</td>
<td>2</td>
<td>0.16</td>
<td>46.5</td>
<td>1286.2</td>
<td>Phenyl propanoid 2</td>
</tr>
<tr>
<td>X1</td>
<td>3</td>
<td>0.27</td>
<td>92.8</td>
<td>3000.6</td>
<td>Phenyl propanoid 3</td>
</tr>
<tr>
<td>X1</td>
<td>4</td>
<td>0.40</td>
<td>14.8</td>
<td>349.1</td>
<td>Phenyl propanoid 4</td>
</tr>
<tr>
<td>X1</td>
<td>5</td>
<td>0.50</td>
<td>40.2</td>
<td>953.0</td>
<td>Phenyl propanoid 5</td>
</tr>
<tr>
<td>X1</td>
<td>6</td>
<td>0.98</td>
<td>11.0</td>
<td>140.5</td>
<td>Unknown</td>
</tr>
<tr>
<td>X2</td>
<td>1</td>
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<td>19.4</td>
<td>358.3</td>
<td>Phenyl propanoid 1</td>
</tr>
<tr>
<td>X2</td>
<td>2</td>
<td>0.16</td>
<td>53.3</td>
<td>1423.6</td>
<td>Phenyl propanoid 2</td>
</tr>
<tr>
<td>X2</td>
<td>3</td>
<td>0.27</td>
<td>85.0</td>
<td>3123.3</td>
<td>Phenyl propanoid 3</td>
</tr>
<tr>
<td>X2</td>
<td>4</td>
<td>0.36</td>
<td>10.4</td>
<td>209.7</td>
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</tr>
<tr>
<td>X2</td>
<td>5</td>
<td>0.41</td>
<td>15.4</td>
<td>279.3</td>
<td>Phenyl propanoid 5</td>
</tr>
<tr>
<td>X2</td>
<td>6</td>
<td>0.51</td>
<td>26.7</td>
<td>820.4</td>
<td>Phenyl propanoid 6</td>
</tr>
<tr>
<td>X2</td>
<td>7</td>
<td>0.97</td>
<td>29.3</td>
<td>704.5</td>
<td>Unknown</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>0.43</td>
<td>124.3</td>
<td>4570.5</td>
<td>Eugenol standard</td>
</tr>
</tbody>
</table>

On the other hand, the methanol extract of *L. longiflorus* leaf sample collected from *F. religiosa* host tree showed 7 compounds (Table 1X2; Figure 1b-ii) with peak $R_f$ values ranging from (0.06 to 0.97, peak height from 10.4 to 85.0 and peak area from 209.7 to 3123.3 as compared to eugenol standard (0.43, 124.3 and 4570.5, respectively) and out of 7 compounds, 6 were identified as phenyl propanoid compounds (peak no. 1-6) and other one was unknown.

HPTLC analysis for phenyl propanoid 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 2; Figure 2b) and were compared with eugeol standard.
The methanol extract of *L. longiflorus* bark samples (Y1) collected from *C. equisetifolia* host tree showed 8 compounds (Table 1Y1; Figure 3b-i) with varied peak R\(_f\) values (0.19-0.96), peak height (21.6-91.4) and peak area (416.1-2700.8) as compared to eugenol standard (0.56, 99.2 and 4650.5, respectively). Out of 8 compounds detected, 5 compounds (peak no. 1, 3-6) were identified as phenyl propanoids and others were unknown. Similarly, the methanol extract of *L. longiflorus* bark sample collected from *F. religiosa* host tree revealed 6 compounds (Table 2Y2; Figure 2b-ii) with peak R\(_f\) values ranging from 0.21 to 0.97, peak height from 13.7 to 71.8 and peak area from 432.7 to 2677.6 as compared to eugenol standard (0.56, 99.2 and 4650.5, respectively). Among the 6 compounds detected, 3 were identified as phenyl propanoid compounds (peak no. 2-4) and others were unknown (Table 2-Y2; Figure 2b-ii).

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

<table>
<thead>
<tr>
<th>Track sample</th>
<th>Peak</th>
<th>Rf</th>
<th>Height</th>
<th>Area</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>1</td>
<td>0.19</td>
<td>21.9</td>
<td>448.9</td>
<td>Phenyl propanoid 1</td>
</tr>
<tr>
<td>Y1</td>
<td>2</td>
<td>0.21</td>
<td>27.2</td>
<td>416.1</td>
<td>Unknown</td>
</tr>
<tr>
<td>Y1</td>
<td>3</td>
<td>0.29</td>
<td>70.3</td>
<td>2547.4</td>
<td>Phenyl propanoid 2</td>
</tr>
<tr>
<td>Y1</td>
<td>4</td>
<td>0.42</td>
<td>91.4</td>
<td>2700.8</td>
<td>Phenyl propanoid 3</td>
</tr>
<tr>
<td>Y1</td>
<td>5</td>
<td>0.47</td>
<td>32.9</td>
<td>811.3</td>
<td>Phenyl propanoid 4</td>
</tr>
<tr>
<td>Y1</td>
<td>6</td>
<td>0.62</td>
<td>27.3</td>
<td>1013.7</td>
<td>Phenyl propanoid 5</td>
</tr>
<tr>
<td>Y1</td>
<td>7</td>
<td>0.78</td>
<td>21.6</td>
<td>856.8</td>
<td>Unknown</td>
</tr>
<tr>
<td>Y1</td>
<td>8</td>
<td>0.96</td>
<td>67.8</td>
<td>1948.0</td>
<td>Unknown</td>
</tr>
<tr>
<td>Y2</td>
<td>1</td>
<td>0.21</td>
<td>22.8</td>
<td>949.7</td>
<td>Unknown</td>
</tr>
<tr>
<td>Y2</td>
<td>2</td>
<td>0.30</td>
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<td>2677.6</td>
<td>Phenyl propanoid 1</td>
</tr>
<tr>
<td>Y2</td>
<td>3</td>
<td>0.43</td>
<td>52.5</td>
<td>1784.0</td>
<td>Phenyl propanoid 2</td>
</tr>
<tr>
<td>Y2</td>
<td>4</td>
<td>0.62</td>
<td>13.7</td>
<td>650.3</td>
<td>Phenyl propanoid 3</td>
</tr>
<tr>
<td>Y2</td>
<td>5</td>
<td>0.79</td>
<td>29.7</td>
<td>432.7</td>
<td>Unknown</td>
</tr>
<tr>
<td>Y2</td>
<td>6</td>
<td>0.97</td>
<td>57.0</td>
<td>1373.3</td>
<td>Unknown</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>0.56</td>
<td>99.2</td>
<td>4650.5</td>
<td>Eugenol standard</td>
</tr>
</tbody>
</table>

The leaf (X1) and bark (Y1) samples of *L. longiflorus* from *C. equisetifolia* host tree showed no similar peak R\(_f\) values in the compounds detected (Tab.1X1 & Tab.-2Y1), while the leaf and bark samples of *L. longiflorus* from *F. religiosa* host tree showed one compound (peak no. 7 of X2 and 6 of Y2) similar in their peak R\(_f\) values (0.97) (Tab.1X2 & Tab.-2Y2).

However, the three phenyl propanoid compounds (peak no. 2 & 3 of X1 and peak no. 2 & 3 of X2) of the *L. longiflorus* leaf samples collected from *C. equisetifolia* and *F. religiosa* host
trees showing same peak R_f values (0.16 & 0.27, respectively) (Table 1). On the other hand, the bark samples (Y1 & Y2) of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees showing two identical phenyl propanoid compounds (peak no. 2 & 6 of Y1 and 1 & 4 of Y2) with similar peak R_f values (0.21 & 0.62) (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 phenyl propanoid compounds and the host trees influenced on the nature and number of phenyl propanoids present in the hemiparasitic plants.

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