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HPTLC ANALYSIS OF GLYCOSIDES PROFILE IN THE LEAF AND BARK SAMPLES OF *LORANTHUS LONGIFLORUS* DESR COLLECTED FROM TWO HOST TREES.

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ABSTRACT

HPTLC determinations of glycoside compound profile was carried out in the methanolic 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 *L. longiflorus* leaf samples obtained from *C. equisetifolia* and *F. religiosa* host trees showed 7 and 6 compounds, respectively, and were compared with stevioside standard. Among the compounds, 5 and 4 compounds in each sample, respectively, was identified as glycosides while other compounds were unknown. Three compounds from each *L. longiflorus* leaf samples collected from *C. equisetifolia* (peak no. 2, 6 & 7) and *F. religiosa* (peak no. 1, 3 & 4) host trees showed similar R_f values (0.12, 0.80 & 0.86, respectively). On the other hand, the methanol extract of *L. longiflorus* bark sample collected from *C. equisetifolia* and F. religiosa host trees contained 17 and 14 compounds in each sample, respectively and were compared with stevioside standard. Among the compounds, 6 compounds, in each sample, was identified as glycosides while other compounds were unknown and 3 compounds from both bark samples obtained from *C. equisetifolia* (peak no. 4, 9 & 14) and F. religiosa (peak no. 4, 9, & 14) showed similar R_f values (0.22, 0.59 & 0.77). None of the compounds from leaf and park samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* showed 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 glycosides and the host trees influenced on the nature and number of glycosides present in the hemiparasitic plants.

KEYWORDS: Hemiparasite, *Loranthus longiflorus*, Leaf sample, bark sample, methanol extracts, *Casuarina equisetifolia* host, *Ficus religiosa* host, HPTLC analysis, Glycoside profile.

INTRODUCTION

The use of herbal medicine for the treatment of diseases and infections is as old as mankind. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites.[1,2] They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Among the secondary metabolites, glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides and these can be activated by enzyme hydrolysis.[3] which causes the sugar part to be broken off, making the chemical available for use. Glycosides are widely used in pharmaceutical industries including production of steroidal hormones, vitamins, active pharmaceutical ingredients and also for therapeautic purposes such as conjustive heart failure (digitalis), cardiotonic and diuretic, rodenticide, anticancer, laxative and purgative, anti-inflammatory, emulsifier, expectorant, liver disorders, etc. 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.[4,6] Medicinal properties of this hemiparasite may vary in effects respective to different hosts it establishes a relation with.[7,8] The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the glycoside compound profile in the leaf/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/bark samples (5g) from *C. equisetifolia* and *F. religiosa* host trees were extracted with Methanol in Soxhlet apparatus for 3h. 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 glycoside 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.[9]

HPTLC analysis for glycosides

- **Test solution**: Methanol extracts of *L. longiflorus* leaf/bark samples obtained from *C. equisetifolia* and *F. religiosa* host trees.
- **Standard solution**: Methanol.
- **Standard chemical**: STE – Stevioside was used as reference standard compound.
- **Mobile phase**: Ethyl acetate-Ethanol-Water (8: 2: 1.2).
- **Spray reagent**: Chloramines-T reagent.

RESULTS AND DISCUSSION

HPTLC analysis of *Loranthus longiflorus* leaf sample
The chromatogram (Figure: 1a) shows glycoside profile of methanol extract of *L. longiflorus* leaf (X) samples collected from *C. equisetifolia* (Figure: 1a-i) and F. religiosa (Figure: 1a-ii) host trees and is compared with stevioside (STE) standard. Blue-yellow coloured fluorescent zones present in the stevioside standard and plant samples tracks at UV 366nm mode were observed in the chromatogram after derivatization and this confirmed the presence of glycoside compounds in the leaf and bark samples of *L. longiflorus*.

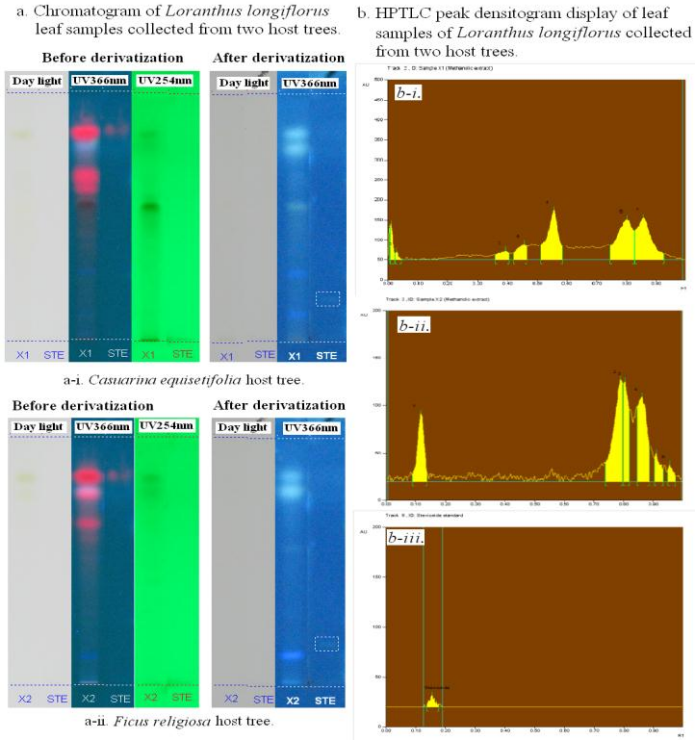

Figure 1: Chromatogram (a) and peak densitogram (b) shows glycoside profile in the *Loranthus longiflorus* leaf samples collected from *Casuarina equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; STE-Stevioside standard - b-iii).

The densitogram (Figure: 1b) shows the profile of glycoside compounds present in the methanol extract of *L. longiflorus* leaf (X) samples collected from *C. equisetifolia* (Figure: 1b-i) and *F. religiosa* (Figure: 1b-

ii) host trees; and stevioside standard for leaf (Figure: 1b-iii) and samples scanned at 366nm.

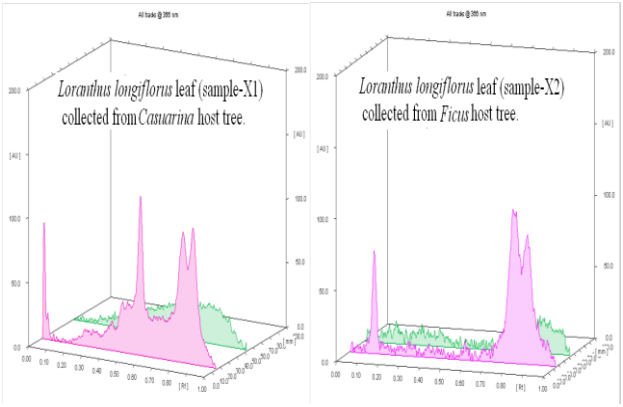

Figure 2: HPTLC-3D display of densitogram showing all tracks –*L. longiflorus* leaf samples (X1/ X2) and standard (stevioside-green coloured) scanned at 366nm.

The 3D display of densitogram for glycoside profile shows all tracks of *L. longiflorus* plant leaf samples (X1/X2) collected from *C. equisetifolia* (X1) and *F. religiosa* (X2) host trees and stevioside standard scanned at 366nm for leaf samples (Figure: 2).

HPTLC analysis for glycoside 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 stevioside standard.

The methanol extract of *L. longiflorus* leaf samples (X1) obtained from *Casuarina equisetifolia* host trees showed 7 compounds (Table: 1X-1; Figure: 1b-i) with peak R_f values ranging from 0.01 to 0.86, peak height ranging from 20.0 to 124.0 and peak area ranging from 206.0 to 4854.6 as compared to stevioside standard (0.16, 11.7 and 295.5, respectively). Among the 7 compounds detected, 5 were identified as glycosides (peak no. 2, 6 & 7) and the others were unknown.

On the other hand, the methanol extract of *L. longiflorus* leaf sample collected from *F. religiosa* host tree showed 6 compounds (Table: 2X-2; Figure: 3b-ii) with peak R_f values ranging from (0.12 to 0.95, peak height from 18.1 to 107.5 and peak area from 266.1 to 2850.6 as compared to stevioside standard (0.16, 11.7 and 295.5, respectively) and out of 6 compounds, 4 were identified as glycosides and others were unknown.

Table 1: Peak table for HPTLC analysis of glycosides 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	0.01	90.1	791.1	Unknown
X1	**2**	**0.12**	**20.0**	**206.0**	**Glycosid1**
X1	3	0.28	22.7	695.7	Glycoside2
X1	4	0.46	39.0	1153.2	Unknown
X1	5	0.56	124.0	4192.2	Glycoside3
X1	**6**	**0.80**	**100.9**	**4854.6**	**Glycosid4**
X1	**7**	**0.86**	**105.6**	**4780.6**	**Glycosid5**
X2	**1**	**0.12**	**71.6**	**1401.1**	**Glycosid1**
X2	2	0.79	107.5	2850.6	Glycosid2
X2	**3**	**0.80**	**105.7**	**1481.5**	**Glycosid3**
X2	**4**	**0.86**	**90.1**	**2607.6**	**Glycosid4**
X2	5	0.91	30.4	520.5	Unknown
X2	6	0.95	18.1	266.1	Unknown
Control	**1**	**0.16**	**11.7**	**295.5**	**Stevioside standard**

HPTLC analysis of *Loranthus longiflorus* bark sample

The chromatogram (Figure: 3a) shows glycoside profile of methanol extract of *L. longiflorus* bark (Y) samples collected from *C. equisetifolia* (Figure: 3a-i) and F. religiosa (Figure: 3a-ii) host trees and is compared with stevioside (STE) standard. Blue-yellow coloured fluorescent zones present in the stevioside standard and plant samples tracks at UV 366nm mode were observed in the chromatogram after derivatization and this confirmed the presence of glycoside compounds in the leaf and bark samples of *L. longiflorus*.

The densitogram (Figure: 3b) shows the profile of glycoside compounds present in the methanol extract of *L. longiflorus* bark (Y) samples collected from *C. equisetifolia* (Figure: 3b-i) and *F. religiosa* (Figure: 3b-ii) host trees; and stevioside standard for bark (Figure: 3b-iii) samples scanned at 366nm.

The 3D display of densitogram for glycoside profile shows all tracks of *L. longiflorus* plant bark samples (Y1/Y2) collected from *C. equisetifolia* (Y1) and *F. religiosa* (Y2) host trees and stevioside standard scanned at 366nm for leaf samples (Figure: 4).

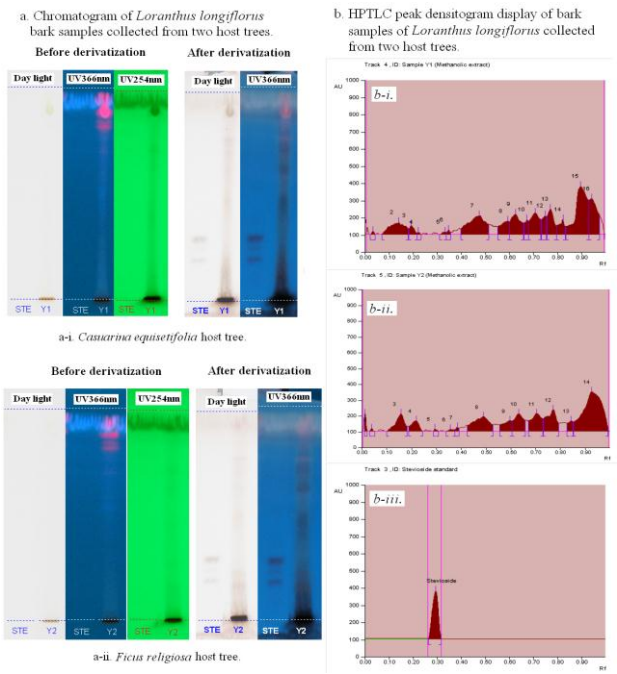

Figure 3: Chromatogram (a) and peak densitogram (b) shows glycoside profile in the *Loranthus longiflorus* bark samples collected from *Casuarina equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (Y1/Y2-sample code; STE -Stevioside standard - b-iii).

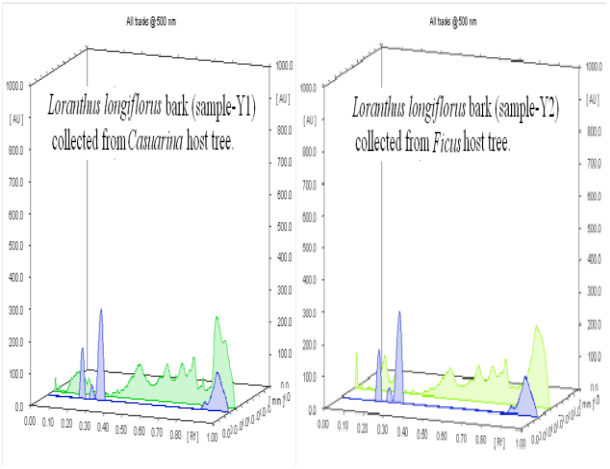

Figure 4: 3D display of densitogram showing all tracks –*Loranthus longiflorus* bark samples (Y1/ Y2) and standard (Stevioside-blue coloured) scanned at 500nm.

HPTLC analysis for glycoside 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) and were compared with stevioside standard.

Table 2: Peak table for HPTLC analysis of glycosides 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	0.03	15.3	132.4	Unknown
Y1	2	0.14	69.1	4044.3	Glycoside1
Y1	3	0.19	51.6	923.1	Unknown
Y1	**4**	**0.22**	**11.4**	**102.9**	**Unknown**
Y1	5	0.33	11.9	165.4	Unknown
Y1	6	0.35	24.9	248.9	Unknown
Y1	7	0.37	20.6	270.0	Unknown
Y1	8	0.47	104.3	6696.4	Glycoside2
Y1	**9**	**0.59**	**68.8**	**2166.4**	**Unknown**
Y1	10	0.62	107.0	3808.5	Glycoside3
Y1	11	0.67	73.1	1076.9	Unknown
Y1	12	0.71	110.3	3699.0	Glycoside4
Y1	13	0.75	92.7	1608.9	Unknown
Y1	**14**	**0.77**	**131.8**	**2946.4**	**Glycoside5**
Y1	15	0.82	69.8	1366.1	Unknown
Y1	16	0.90	259.6	10849.6	Glycoside6
Y1	17	0.94	187.2	5471.3	Unknown
Y2	1	0.01	110.5	554.0	Unknown
Y2	2	0.04	17.1	125.3	Unknown
Y2	3	0.16	109.6	3457.6	Glycoside1
Y2	**4**	**0.22**	**66.3**	**1871.0**	**Glycoside2**
Y2	5	0.29	14.3	115.4	Unknown
Y2	6	0.36	12.7	231.0	Unknown
Y2	7	0.38	24.4	243.1	Unknown
Y2	8	0.49	93.2	5411.2	Glycoside3
Y2	**9**	**0.59**	**66.2**	**2163.1**	**Unknown**
Y2	10	0.63	110.5	3746.0	Glycoside4
Y2	11	0.70	112.5	4251.6	Glycoside5
Y2	**12**	**0.77**	**136.6**	**4899.5**	**Glycoside6**
Y2	13	0.84	67.0	1235.8	Unknown
Y2	14	0.93	251.0	17175.9	Unknown
Control	**1**	**0.29**	**282.0**	**6513.8**	**Stevioside standard**

The methanol extract of *L. longiflorus* bark samples (Y1) collected from *C. equisetifolia* host tree showed 17 compounds (Table: 2Y-1; Figure: 3b-i) with varied peak R_f values (0.03-0.94), peak height (11.4-259.6) and peak area (102.9-10849.6) as compared to stevioside standard (0.29, 282.0 and 6513.8, respectively). Out of 17 compounds detected, 6 compounds (peak no. 2, 8, 10, 12, 14 &16) were identified as glycosides and others were unknown. Similarly, the methanol extract of *L. longiflorus* bark (Y) sample collected from *F. religiosa* host tree revealed 14 compounds (Table: 2Y-2; Figure: 3b-ii) with peak R_f values ranging from 0.01 to 0.93, peak height from 12.7 to 251.0 and peak area from 125.3 to 17175.9 as compared to standard stevioside (0.29, 282.0 and 6513.8, respectively). Among the 14 compounds detected, 6 were identified as glycosides (peak no. 3, 4, 8, 10, 11 & 12) and others were unknown.

The leaf (X1/X2) and bark (Y1/Y2) samples of *L. longiflorus* from *C. equisetifolia* and *F. religiosa* host tree showed no similar peak R_f values in the compound detected. But, the three glycoside compounds (peak no. 2, 6 & 7 of X1 and peak no. 1, 3, & 4 of X2) of the leaf samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees showing (Table: 1) same peak R_f values (0.12, 0.80 & 0.86, respectively). Similarly, the bark samples (Y1/Y2) of *L. longiflorus* collected from *C. equisetifolia* and F. religiosa host trees showing (Table: 2) three identical glycoside compounds (peak no. 4, 9 & 14 of Y1 and 4, 9 & 12 of Y2) with similar peak R_f values (0.22, 0.59 & 0.77).

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 glycosides and the host trees influenced on the nature and number of glycosides present in the hemiparasitic plants.

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