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HPTLC DETERMINATION OF PHENOLIC ACIDS IN *LORANTHUS LONGIFLORUS* DESR (A HEMIPARASITE) COLLECTED FROM TWO HOST TREES

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Abstract

Influence of host plants on the phenolic acids profile of *Loranthus longiflorus* leaf and bark samples collected from *Casuarina equisetifolia* and *Ficus religiosa* host trees were determined by HPTLC method. The methanolic extract of *L. longiflorus* leaf samples obtained from *C. equisetifolia* host trees showed 13 compounds while, it was 8 compounds in the leaf samples collected from *F. religiosa* host tree and among the compounds detected, 4 and 2 compounds in each sample was identified as phenolics, respectively, whereas the others were unknown. Among the 2 identified phenolic compounds in *L. longiflorus* leaf from *F. religiosa*, one compound (peak no. 5) showed similar peak R_f value (0.66) with the quercetin standard. The methanolic extract of *L. longiflorus* bark samples collected from *C. equisetifolia* and *F. religiosa* host trees contained each 9 compounds and of which, 4 compounds from each host were identified as phenolics while others were unknown. Two compounds of leaf (peak no. 8/13 & 4/8) and three compounds of bark (peak no. 1/2/8 & 1/2/8) samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host tree, respectively, shows similar R_f values (54/95 & 0.01/0.12/0.69, respectively). Two compounds (peak no. 12/2) and bark (peak no. 9/3) from leaf and bark samples of *L. longiflorus* from *C. equisetifolia* and *F. religiosa* showed similar R_f values (95/22), respectively.

Key words : Phenolic acids, Leaf/bark methanol extracts, *Loranthus longiflorus,* Hemiparasite, *Casuaria equisetifolia* host, *Ficus religiosa* host.

Introduction

Phenols are very important plant constituents because of their radical scavenging ability due to the hydroxyl groups (Hatano *et al*., 1989). Phenolic acid compounds seem to be universally distributed in plants. The importance of antioxidant activities of phenolic compounds against oxidative damage diseases and their possible usage in processed foods as a natural antioxidant has reached an elevated level in recent years. Polyphenols are known to exhibit a variety of biological actions such as free radical scavenging, metal chelation, modulation of enzyme activity and more recently to effect signal transduction, activation of transcription factors and gene expression (Bito *et al*., 2000). Angiospermic hemiparasitic plant *L. longiflorus* reported to contain biologically active substances (Kacharu and Krishnan, 1979; Rastogi and Mehotra, 1993; Ramachandran and Krishnakumary, 1999; Chandrakasan and Neelamegam, 2011; 2012). *L. parasiticus* reported to possess the highest antioxidant capacities and total phenolic content among 50 plants tested, and could be rich potential source of natural antioxidants (Ren-You Gan *et al*., 2011). The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the phenolic acids profile in the leaf and bark samples of *Loranthus longiflorus* Desr. [Syn.–*Dendrophthoe falcata* (L.f.) Ettingsh], a hemiparasite.

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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

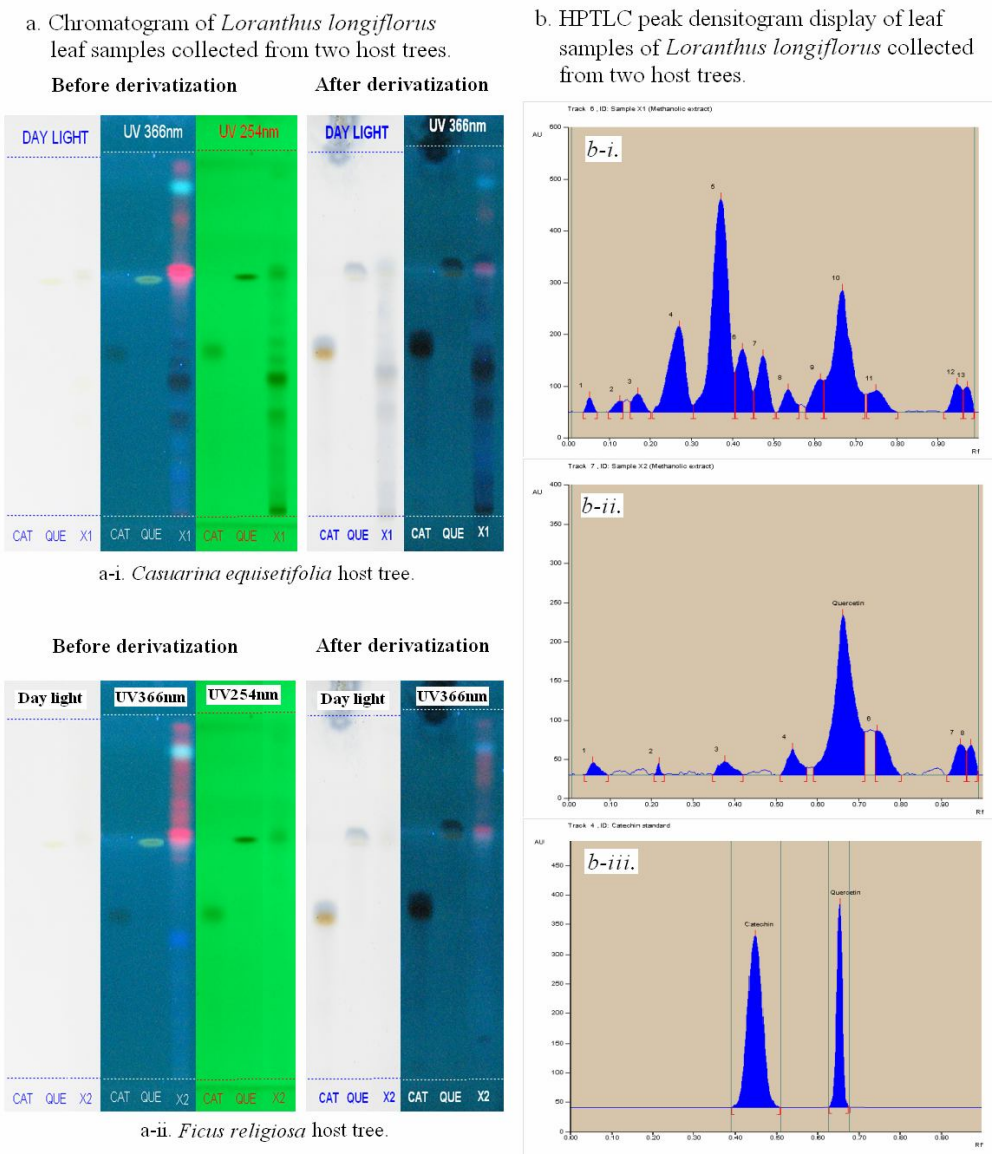

Fig. 1 : Chromatogram (a) and peak densitogram (b) shows phenolics profile in the *Loranthus longiflorus* leaf samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; CAT-Catechin & QUE-Quercetin standards (b-iii).

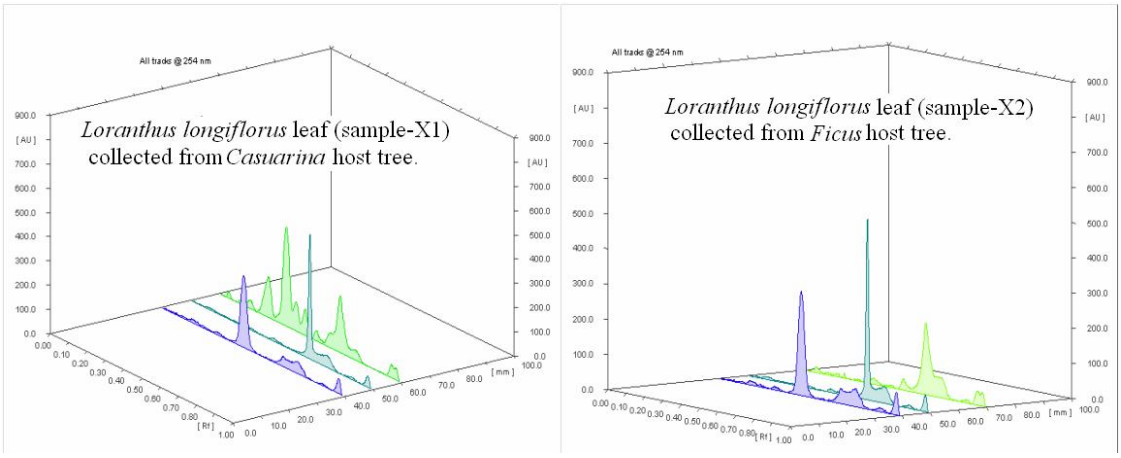

Fig. 2 : HPTLC-3D display of densitogram showing all tracks –plant leaf samples (X1/ X2- light green colour) and standard (Catechin-blue coloured; Quercetin –dark green colour) scanned at 254nm.

Table 1 : Peak table for HPTLC analysis of phenolic compounds 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	0.05	28.2	386.5	Unknown
X1	2	0.13	21.6	419.8	Unknown
X1	3	0.17	35.8	868.6	Unknown
X1	4	0.27	165.5	6056.9	Phenolic 1
X1	5	0.37	411.9	14770.7	Unknown
X1	6	0.42	121.5	3233.5	Phenolic 2
X1	7	0.47	109.1	2642.4	Unknown
X1	**8**	**0.54**	**44.0**	**1071.4**	**Phenolic 3**
X1	9	0.61	63.4	1556.7	Unknown
X1	10	0.67	235.4	9250.8	Phenolic 4
X1	11	0.75	41.7	1548.6	Unknown
X1	12	0.95	54.1	1149.8	Unknown
X1	**13**	**0.97**	**48.8**	**831.8**	**Unknown**
X2	1	0.06	15.7	342.2	Unknown
X2	2	0.22	14.6	112.1	Unknown
X2	3	0.38	17.2	560.6	Unknown
X2	**4**	**0.54**	**33.0**	**870.6**	**Phenolic 1**
X2	5	0.66	203.8	8707.7	Phenolic 2 (**Quercetin**)
X2	6	0.74	56.7	1583.1	Unknown
X2	7	0.94	39.2	898.1	Unknown
X2	**8**	**0.97**	**38.0**	**643.2**	**Unknown**
Control	**1**	**0.45**	**293.8**	**10052.0**	**Catechin standard**
Control	**2**	**0.66**	**514.3**	**8113.7**	**Quercetin standard**

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 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 was uses as standard solution. 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 phenolic compounds. CAT – Catechin and QUE – Quercetin were used as reference standard compounds and the Toluene-Acetone-Formic acid (4.5: 4.5: 1) used as mobile phase. Folin Cio-Calteu was used as spray reagent.

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 (Shah *et al*., 2008).

Results and Discussion

HPTLC analysis for carotenoid 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 tables 1 and 2. The chromatogram (figs. 1a & 3a) shows phenolic profile of methanolic extract of *L. longiflorus* leaf (X) and bark (Y) samples collected from *C. equsetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees and compared with catechin (CAT) and quercetin (QUE) standards. Blue coloured zones at day light mode

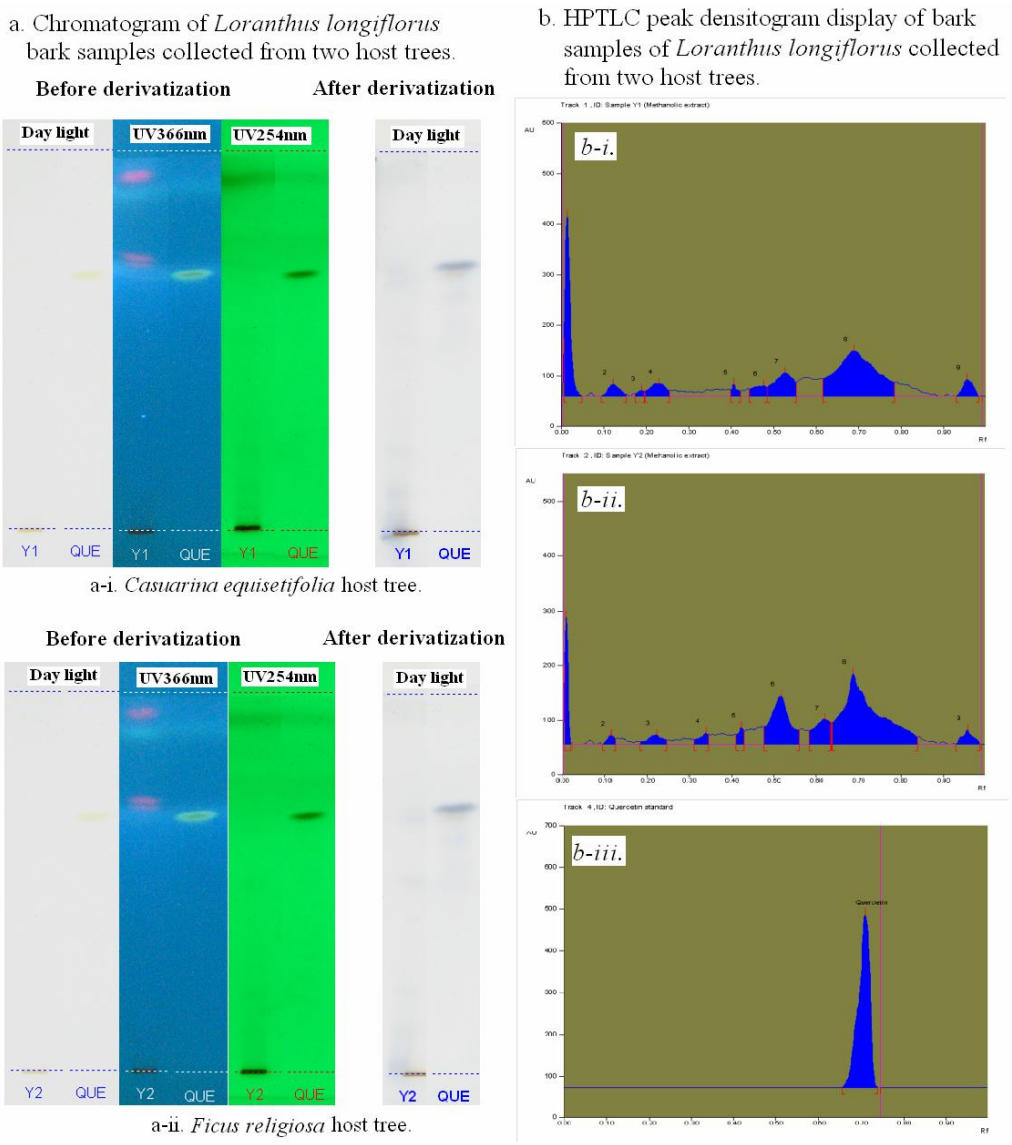

Fig. 3 : Chromatogram (a) and peak densitogram (b) shows phenolic acids profile in the *L. longiflorus* bark samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; QUE-Quercetin standard (b-iii).

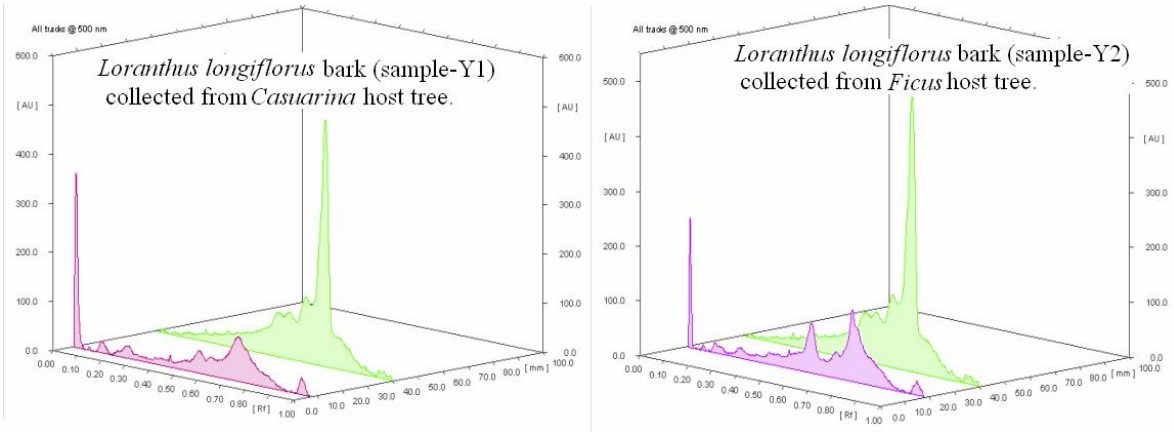

Fig. 4 : 3D display of densitogram showing all tracks –*Loranthus longiflorus* bark samples (Y1/ Y2) and standard (Quercetin-green coloured) scanned at 500nm.

Table 2 : Peak table for HPTLC analysis of phenolic compounds 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**	**0.01**	**356.4**	**4072.3**	**Unknown**
Y1	**2**	**0.12**	**23.4**	**599.2**	**Phenolic 1**
Y1	3	0.19	10.9	159.4	Unknown
Y1	4	0.23	25.2	903.5	Phenolic 2
Y1	5	0.41	24.0	287.4	Unknown
Y1	6	0.48	21.3	618.7	Unknown
Y1	7	0.53	46.2	1868.9	Phenolic 3
Y1	**8**	**0.69**	**89.7**	**7762.4**	**Phenolic 4**
Y1	9	0.95	32.9	740.5	Unknown
Y2	**1**	**0.01**	**235.3**	**1691.4**	**Unknown**
Y2	**2**	**0.12**	**17.3**	**263.9**	**Phenolic 1**
Y2	3	0.22	18.5	641.1	Phenolic 2
Y2	4	0.34	21.7	453.6	Unknown
Y2	5	0.42	32.0	422.8	Unknown
Y2	6	0.52	90.1	3623.6	Phenolic 3
Y2	7	0.62	47.1	1689.6	Unknown
Y2	**8**	**0.69**	**130.0**	**9542.3**	**Phenolic 4**
Y2	9	0.96	27.4	630.0	Unknown
Control	**1**	**0.71**	**502.1**	**20660.8**	**Quercetin standard**

present in the catechin and quercetin standards and plant samples tracks at day light/UV 366nm mode were observed in the chromatogram after derivatization and this confirmed the presence of phenolic compounds in the leaf and bark samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees.

The densitogram shows the profile of phenolics compounds (such as number of peaks, peak R_f values, peak height, peak area and the known and unknown compounds) present in the methanolic extract of *L. longiflorus* leaf (table 1; figs. 1b-i & 1b-ii) and bark (table 2, figs. 3-bi & 3b-ii) samples collected from *C. equisetifolia* (figs. 1b-i & 3b-i) and *F. religiosa* (figs. 3b-i & 3 b-ii) host trees; and quercetin standard for leaf (fig. 1b-iii) and bark (fig. 3b-iii) samples scanned at 254nm and 500nm, respectively.

The 3D display of densitogram for phenolic profile (figs. 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 standards catechin and quercetin for leaf (X1/X2) and quercetin for bark (Y1/Y2) samples scanned at 254nm (fig. 2) and 500nm (fig. 4), respectively.

The methanolic extract of *L. longiflorus* leaf samples (X1) obtained from *C. equisetifolia* host trees showed 13 compounds (table 1; X1; fig. 1b-i) with peak R_f values ranging from 0.01 to 0.97, peak height ranging from 21.6 to 411.9 and peak area ranging from 386.5 to 14770.7 as compared to quercetin standard (0.66, 514.3 and 81137, respectively). Among the 13 compounds detected, 4 were identified as phenolics (peak no. 4, 6, 8 & 10) and the others were unknown.

On the other hand, the methanolic extract of *L. longiflorus* leaf sample collected from *F. religiosa* host tree showed 8 compounds (table 1; X2; fig. 1b-ii) with peak R_f values ranging from (0.06 to 0.97, peak height from 14.6 to 203.8 and peak area from 112.1 to 8707.7 as compared to quercetin standard (0.66, 514.3 and 8113.7, respectively) and out of 8 compounds, 2 were identified as phenolics and others were unknown. Among the 2 identified phenolic compounds, one compound (peak no. 5) showed similar peak R_f value (0.66) with the quercetin standard and so it was determined as quercetin.

The methanolic extract of *L. longiflorus* bark samples (Y1) collected from *C. equisetifolia* host tree showed 9 compounds (table 2; Y1; fig. 3b-i) with varied peak R_f values (0.01-0.95), peak height (10.9-356.4) and peak area (159.4-7762.4) as compared to quercetin standard (0.71, 502.1 and 20660.8, respectively). Out of 9 compounds detected, 4 compounds (peak no. 2, 4, 7, & 8) were identified as phenolics and others were unknown.

Similarly, the methanolic extract of *L. longiflorus* bark sample collected from *F. religiosa* host tree revealed 9 compounds (table 2; Y2; fig. 3b-ii) with peak R_f values ranging from 0.01 to 0.96, peak height from 17.3 to 235.3 and peak area from 263.9 to 9542.3 as compared to standard quercetin standard (0.71, 502.1 and 20660.8, respectively). Among the 9 compounds detected, 4 were identified as phenolics (peak no. 2, 3, 6 & 8) and others were unknown.

The leaf (X1) and bark (Y1) samples of *L. longiflorus* from *C. equisetifolia* host tree shows one similar compound (peak no. 12 & 9, respectively) with peak R_f value of 0.95 (tables 1 & 2), and *F. religiosa* host tree also showed one similar phenolic compound (peak no. 2 & 3) with same peak R_f value of 0.22 (tables 1 & 2).

In general, the one phenolic compound (peak no. 8 in

X1 and peak no. 4 in X2) of the leaf samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees shows same peak R_f values (0.54). On the other hand, the bark samples (Y1 & Y2) of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees show three identical phenolic compounds (peak no. 1, 2 & 8 of Y1 and 1, 2 & 8 of Y2) with similar peak R_f values (0.01, 0.12 & 0.69) (tables 1 & 2).

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