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HPTLC EVALUATION OF ALKALOIDS IN *LORANTHUS LONGIFLORUS* DESR. A HEMIPARASITE, COLLECTED FROM TWO HOST TREES

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Abstract

HPTLC determination of alkaloid 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 methanolic extract of *L. longiflorus* leaf samples obtained from *C. equisetifolia* and *F. religiosa* host trees showed 9 and 5 compounds, respectively. Of which, one compound (peak no. 7) was identified as alkaloid in the leaf sample of *L. longiflorus* from *C. equisetifolia* while the others were unknown. Similarly, the methanolic extract of *L. longiflorus* bark sample collected from *C. equisetifolia* and *F. religiosa* host trees showed 5 and 4 compounds, respectively, of which one in each sample (peak no. 4 and 2, respectively) is identified as alkaloid. Two compounds in leaf (peak no. 2/8 & 1/4) and bark (peak no. 5 & 4) samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees shows similar R_f values (0.06/0.65 & 0.88/0.88), respectively. There is no similarity between the compounds detected in the leaf ad bark samples of *L. longiflorus* from *C. equistifolia* and *F. religiosa* host trees.

Key words : Alkaloids, leaf/bark methanol extracts, *Loranthus longiflorus,* hemiparasite, *Casuaria equisetifolia* host, *Ficus religiosa* host.

Introduction

Alkaloids are a group of nitrogen-containing bases, most of which are drugs. Only a few (like caffeine) are derived from purines or pyrimidines, while majority is produced from amino acids. Alkaloid-containing plants have been used by human beings since ancient times for therapeutic and recreational purposes. More than 15,000 naturally occurring alkaloids (mostly of herbal origin) have been found so far, and the number is increasing faster and faster. Although, alkaloids often display unspecific biological activity, just this has rarely turned out to be of therapeutic value. Even though only few genuine alkaloids are in use, many alkaloid derivatives are important as drug leads (Newman *et al*., 2007). Many alkaloids are still used in medicine, usually in the form of salts. The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the alkaloid compound profile in the leaf and bark samples of *Loranthus longiflorus* Desr. (Syn.– *Dendrophthoe falcata* (L.f.) Ettingsh), a hemiparasitic plant.

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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 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 used 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 alkaloid compounds. Colchicine (COL) was used as reference standard compound. Ethyl acetate-Methanol-Water (100: 13.5: 10) was used as the mobile phase. Dragendorff's reagent followed by 10% sodium nitrite reagent 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 alkaloid 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 & 2, figs. 1 to 4.

The chromatogram (figs. 1a & 3a) showing alkaloid profile of methanolic extract of *L. longiflorus* leaf (X) and bark (Y) samples collected from *C. equisteifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees and is compared with colchicine (COL) standard. Pink, blue and Black colour zones were detected in UV (366nm and 254nm) before derivatization in the chromatogram. Black coloured quenching zone present in the colchicine standard (before and after derivatization) at 254nm mode, and bright orange and brown coloured zones at day light mode in the *Loranthus* leaf and bark samples taken were observed in the chromatogram after derivatization. This confirmed the presence of alkaloids in the leaf and bark samples of *L. longiflorus*.

Densitogram shows the HPTLC analysis of alkaloid compound profiles, such as number of peaks, peak R_f values, peak height, peak area and the known and unknown compounds in the methanolic extract of *L. longiflorus* leaf (table 1 (X1/X2); fig. 1b) and bark (table 2 (Y1/Y2); fig. 3b) samples from *C. equisetifolia* (figs. 1b-i and 3b-i) and *F. religiosa* (figs. 1b-ii and 3b-ii) host trees; and colchicines standard (figs. 1b-iii and 3b-iii) for leaf and bark samples scanned at 254nm.

The 3D display of densitogram for alkaloid profile shows all tracks of *L. longiflorus* leaf samples (fig. 2; X1 and X2) and bark samples (fig. 4; Y1 & Y2) collected from *C. equisetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees and colchicine standard scanned at 254nm.

The methanolic extract of *L. longiflorus* leaf samples (X1) obtained from *C. equisetifolia* host tree showed nine compounds (table 1 X1; fig. 1b-i) with peak R_f values ranging from 0.03 to 0.89, peak height ranging from 15.3 to 305.0 and peak area ranging from 201.6 to19374.5 as compared to standard (0.35, 486.1 and 11466.9, respectively). Among the nine compounds, one was identified as alkaloid (peak no.7) and the others were unknown. On the other hand, the methanolic extract of *L. longiflorus* leaf sample collected from *F. religiosa* host tree contained five compounds (table 1; X2; fig. 1b-ii) with peak R_f values ranging from 0.06 to 0.90, peak height from 10.8 to 266.1 and peak area from 175.1 to 20443.4 as compared to colchicine standard (0.35, 486.1 and 11466.9, respectively) and all the five compounds were unknown.

The methanolic extract of *L. longiflorus* bark samples collected from *C. equisetifolia* host tree showed five compounds (table 2,Y1; fig. 3b-i) with different peak R_f values (0.39-0.88), peak height (21.4-3,27) and peak area (526.6-24430.6) as compared to colchicine standard (0.36, 555.0 and 12312.3, respectively). Out of five compounds detected, one (No. 4) was identified as alkaloid and the others were unknown.

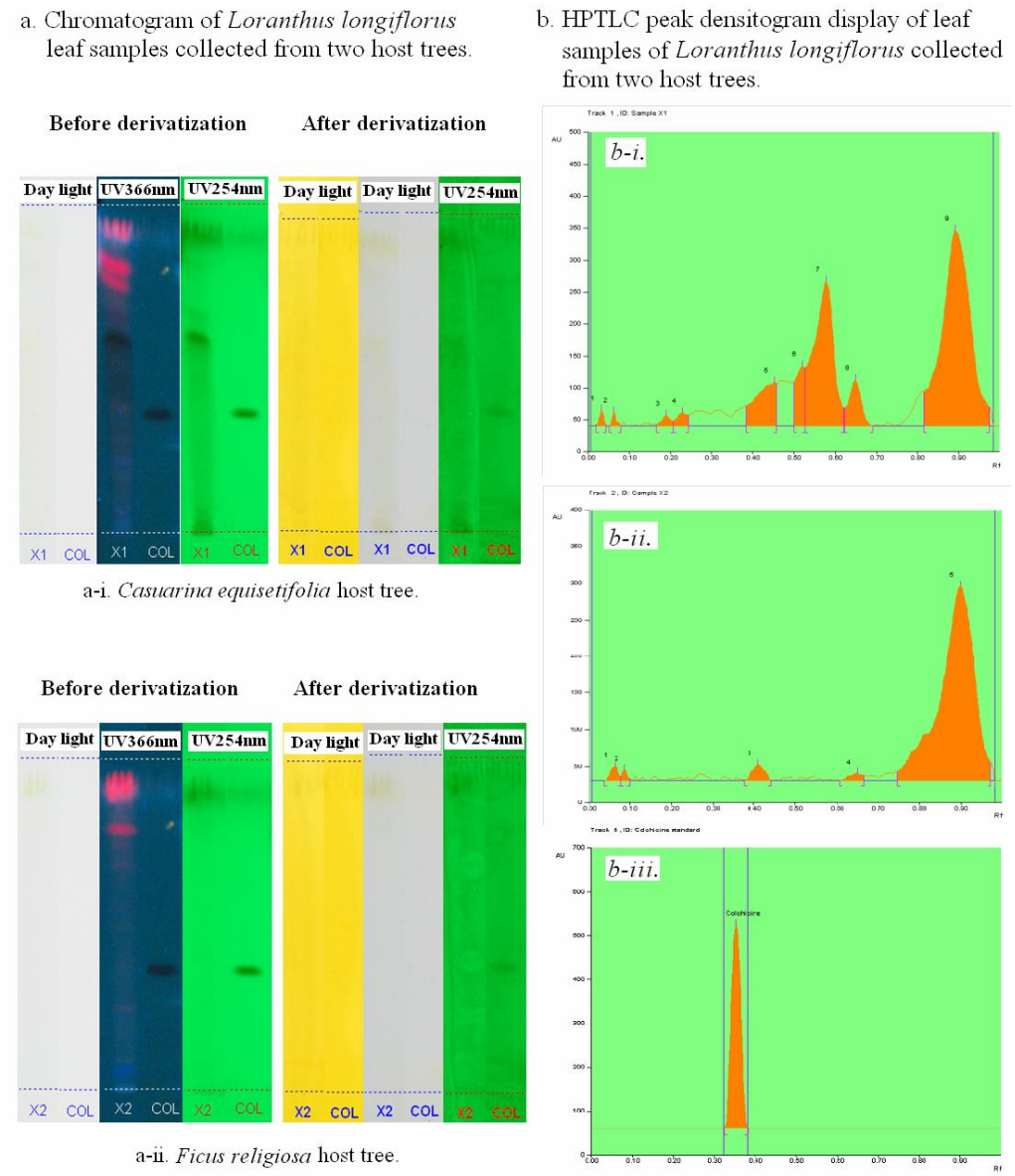

Fig. 1 : Chromatogram (a) and peak densitogram (b) shows alkaloid profile in the *Loranthus longiflorus* leaf samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; COL-Colchicine standard -b-iii).

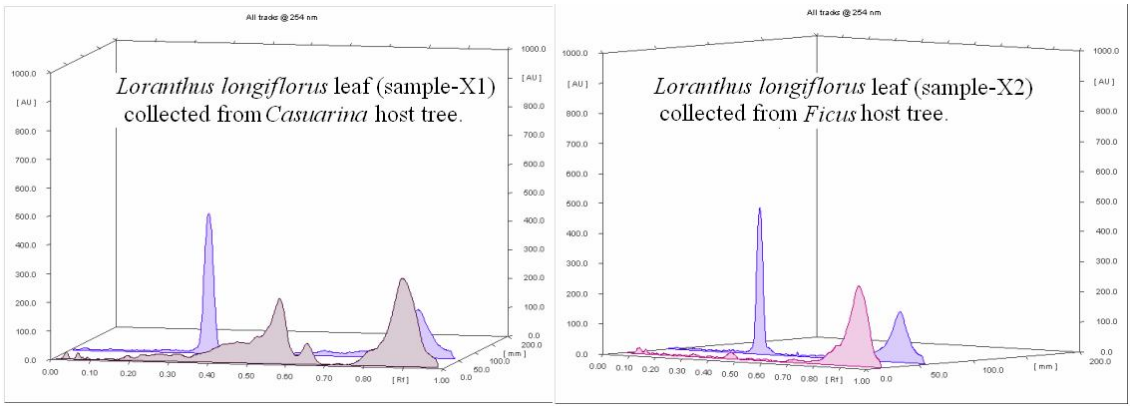

Fig. 2 : HPTLC-3D display of densitogram showing all tracks –plant samples (X1/ X2) and standard (colchicines-blue coloured) scanned at 254nm.

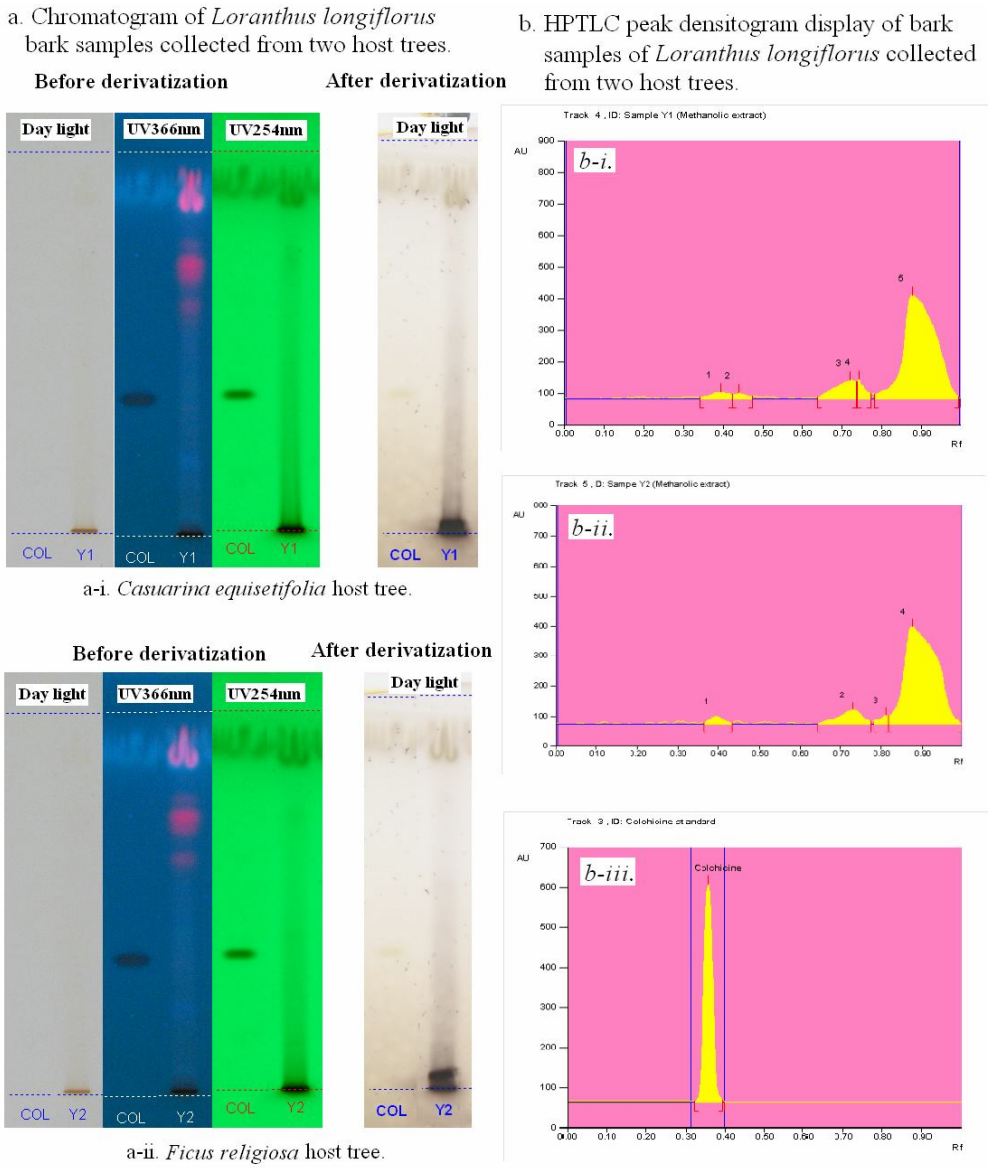

Fig. 3 : Chromatogram (a) and peak densitogram (b) shows alkaloid profile in the *Loranthus longiflorus* bark samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; COL-Colchicine standard -b-iii).

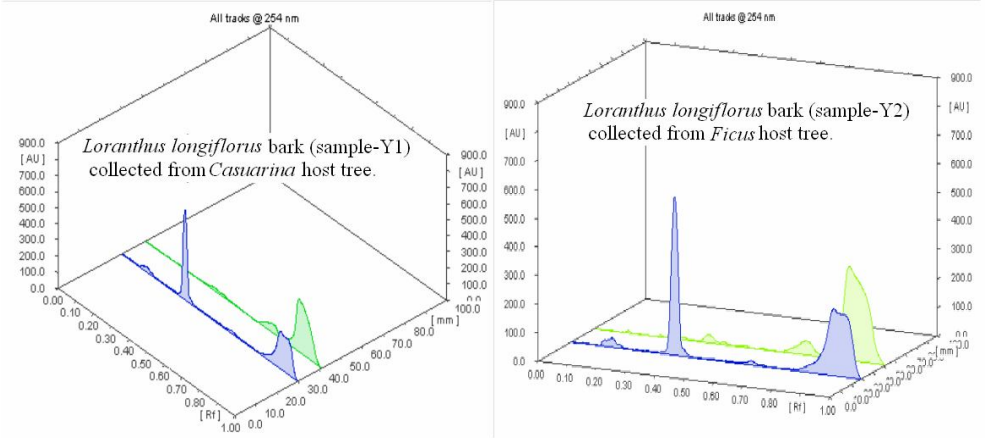

Fig. 4 : HPTLC 3D display of densitogram showing all tracks –*Loranthus longiflorus* bark samples (Y1/Y2) and standard (colchicines-blue coloured) scanned at 254nm.

Table 1 : Peak table for HPTLC analysis of alkaloid profile in the methanol extract of *L. longiflorus* leaf (X1/X2) samples collected from *C. equisetifolia* (X1) and *F. religiosa* (X2) host trees.

Track sample	Peak	Rf	Height	Area	Assigned substance
X1	1	0.03	23.4	225.6	Unknown
X1	**2**	**0.06**	**21.1**	**201.6**	**Unknown**
X1	3	0.19	15.3	308.5	Unknown
X1	4	0.23	20.0	477.1	Unknown
X1	5	0.45	67.6	3128.3	Unknown
X1	6	0.52	92.7	1844.2	Unknown
X1	7	0.58	225.4	9908.4	Alkaloid 1
X1	**8**	**0.65**	**71.3**	**1945.2**	**Unknown**
X1	9	0.89	305.0	19374.5	Unknown
X2	**1**	**0.06**	**19.7**	**353.4**	**Unknown**
X2	2	0.09	15.8	175.1	Unknown
X2	3	0.41	22.1	611.6	Unknown
X2	**4**	**0.65**	**10.8**	**314.7**	**Unknown**
X2	5	0.90	266.1	20443.4	Unknown
Control	**1**	**0.35**	**486.1**	**11466.9**	**Colchicine standard**

Similarly, the methanolic extract of *L. longiflorus* bark sample collected from *F. religiosa* host tree revealed four compounds (table 2,Y2; fig. 3b-ii) with peak R_f values ranging from 0.40 to 0.88, peak height from 29.5 to 328.5 and peak area from 925.6 to 24428.2 as compared to standard colchicine (0.36, 555 and 12312.3, respectively). Among the four compounds detected, one compound (peak no.2) was identified as alkaloid and the remaining were unknown.

The leaf and bark samples of *L. longiflorus* from *C. equisetifolia* (table 1, X1 & table 2, Y1) and *F. religiosa* (table 1, X2 & table 2, Y2) host trees showed no similarities in their compounds detected.

In general, the compounds detected in the leaf samples (table 1) of *L. longiflorus* collected from *C. equistifolia* (X1) and *F. religiosa* (X2) showed 2 compounds (peak no. 2 & 8; peak no. 1 & 4, respectively) similar in their peak R_f values (0.06 & 0.65, respectively). On the other hand, the bark samples (table 2) of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees showed one compound (No. 5 & 4 of samples Y1 & Y2) similar in their peak R_f values (0.88 & 0.88, respectively).

Table 2 : Peak table for HPTLC analysis of alkaloid 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	0.39	21.4	968.1	Unknown
Y1	2	0.44	19.9	526.6	Unknown
Y1	3	0.72	58.7	2846.4	Unknown
Y1	4	0.74	60.5	1049.0	Alkaloid 1
Y1	**5**	**0.88**	**327.1**	**24430.6**	**Unknown**
Y2	1	0.40	29.5	925.6	Unknown
Y2	2	0.73	50.5	2779.4	Alkaloid 1
Y2	3	0.81	32.9	610.2	Unknown
Y2	**4**	**0.88**	**328.5**	**24428.2**	**Unknown**
Control	**1**	**0.36**	**555.0**	**12312.3**	**Colchicine standard**

The alkaloid extracts of plants had showed a strong antioxidant activity, especially a strong radical scavenger power (Maiza-Benabdesselam *et al.*, 2007; Chandrakasan and Neelamegam, 2011; 2012), so they could be used as good sources of natural antioxidants for medicinal and commercial needs. In this study, the HPTLC analysis of methanolic extracts of *L. longiflorus* leaf/bark samples collected from *C. equisetifolia* and *F. religiosa* host trees make certain the presence of alkaloids.

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