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Research Article

HPTLC determination of carotenoid profile in the leaf and bark samples of *loranthus longiflorus* –a hemiparasite, collected from two host trees

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ABSTRACT

Influence of host plants on the carotenoid 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* host trees showed 9 compounds while it was 8 compounds in the leaf samples collected from *F. religiosa* host tree. Among the compounds, 5 and 3 compound in each sample, respectively, was identified as carotinoids while the others were unknown. Four compounds from each leaf samples collected from *C. equisetifolia* (peak no. 4- 6 & 8) and *F. religiosa* (peak no. 1-3 & 6) host trees showed similar R_f values (0.15, 0.19, 0.23 & 0.53, respectively). Similarly, the methanol extract of *L. longiflorus* bark sample collected from *C. equisetifolia* and *F. religiosa* host trees contained 8 compounds each. Of these compounds only 3 from each sample was identified as carotenoids whereas others were unknown and none of these compounds showed any similar R_f values. One compound from leaf and park samples of *L. longiflorus* collected from *C. equisetifolia* (peak no. 6 & 4) and *F. religiosa* (peak no. 4 & 3) showed similar R_f values (0.23 & 0.26), respectively.

Keywords: Carotenoids, Leaf/bark methanol extracts, *Loranthus longiflorus*, Hemiparasite, *Casuarina equisetifolia* host, *Ficus religiosa* host.

INTRODUCTION

Carotenoids are tetraterpenoid organic pigments that occur naturally in the chloroplasts and chromoplasts of plants. There are over 600 known carotenoids includes xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). In human beings, four carotenoids (beta-carotene, alpha-carotene, gamma-carotene, and beta-cryptoxanthin) have vitamin-A activity and can also act as antioxidants. Most of the carotenoids found in foods of people consume have antioxidant activity. Carotenoids are efficient free-radical scavengers and they enhance the vertebrate immune system. People consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of chronic illnesses [1]. The present study is aimed to understand the influence of host trees (*Casuarina equisetifolia* and *Ficus religiosa*) on the carotenoid compound profile in the leaf/bark samples of a hemiparasite -*Loranthus longiflorus* Desr (Syn.–*Dendrophthoe falcata* (L.F.) Ettingsh).

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

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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 carotenoid 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 [2].

HPTLC analysis for carotenoids

- ***Test solution***: Methanol extracts of *L. longiflorus* leaf/bark samples obtained from *C. equisetifolia* and *F. religiosa* host trees.
- ***Standard solution***: Methanol.
- ***Standard chemical***: CRY –Beta Cryptoxanthin (leaf samples) and ZEA - Zeaxanthin (bark samples) were used as reference standard compound.
- ***Mobile phase***: Acetone-Petroleum ether 60°C-80°C (30: 70).
- ***Spray reagent***: Anisaldehyde sulphuric acid reagent.

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 Table -1 and 2. The chromatogram (Fig.-1a & 3a) showing carotenoid profile of methanolic extracts of *L. longiflorus* leaf (X) and bark (Y) samples collected from *C. equisetifolia* (X1/Y1) and *F. religiosa* (X2/Y2) host trees and compared with cryptoxanthin (CRY) standard for leaf samples (X1/X2) and zeaxanthin (ZEA) standard for bark samples (Y1/Y2). Orange coloured fluorescent zones present in the beta-cryptoxanthin and zeaxanthin standards and plant sample track at UV 366nm mode were observed in the chromatogram after derivatization. This confirmed the presence of carotenoids in the leaf and bark samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees.

Densitogram shows the HPTLC analysis of carotenoid compound profiles, (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 (Tab.-1; Fig.-1; X1 & X2) and bark (Tab.-2; Fig.-3; Y1 & Y2) samples from *C. equisetifolia* and *F. religiosa* host trees; and beta-cryptoxanthin standard for leaf (Fig.-1b-iii) and zeaxanthin standard for bark (Fig.-3b-iii) samples scanned at 366nm and 500nm, respectively.

The 3D display of densitogram for carotenoid profile shows all tracks of *L. longiflorus* leaf (X1/X2) and bark (Y1/Y2) samples collected from *C. equisetifolia* (X1&Y1) and *F. religiosa* (X2/Y2) host trees, and standards beta-cryptoxanthin for leaf (X1/X2) and zeaxanthin for bark (Y1/Y2) samples scanned at 366nm (Fig.-2) and 500nm (Fig.-4), respectively.

The methanol extract of *L. longiflorus* leaf samples (X1) obtained from *C. equisetifolia* host trees showed nine compounds (Tab.-1; X1; Fig.1b-i) with peak R_f values ranging from 0.01 to 0.91, peak height ranged from 10.5 to 71.5 and peak area ranging from 228.7 to 1600.2 as compared to cryptoxanthin standard (0.43, 59.0 and 1772.1, respectively). Among the nine compounds detected, 5 were identified as carotenoids (peak no. 2, 3, 4, 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 8 compounds (Tab.-1; X2; Fig.1b-

ii) with peak R_f values ranging from (0.15 to 0.95, peak height from 12.9 to 72.0 and peak area from 328.8 to 2424.9 as compared to cryptoxanthin standard (0.43, 59.0 and 1772.1, respectively) and out of 8 compounds (peak no. 1, 3 & 4), 3 were identified as carotenoids and others were unknown.

The methanolic extract of *L. longiflorus* bark samples (Y1) collected from *C. equisetifolia* host tree showed 8 compounds (Tab.-2,Y1; Fig.-3b-i) with varied peak R_f values (0.08-0.75), peak height (14.1-385.9) and peak area (257.9-31765.1) as compared to zeaxanthin standard (0.68, 400.2 and 15557.0, respectively). Out of 8 compounds detected, three (peak no. 3, 6 & 7) were identified as carotenoids and the others were unknown. Similarly, the methanol extract of *L. longiflorus* bark sample collected from *F. religiosa* host tree revealed 8 compounds (Tab.-2,Y2; Fig.-3b-ii) with peak R_f values ranging from 0.03 to 0.85, peak height from 14.3 to 304.3 and peak area from 151.0 to 38041.2 as compared to standard zeaxanthin (0.68, 400.2 and 15557.0, respectively) (Fig.-3b-iii). Among the 8 compounds detected, 3 compounds (peak no. 3, 6 & 7) were identified as carotenoids and the remaining were unknown (Tab. 2-Y2; Fig.3b-ii).

The leaf (X1) and bark (Y1) samples of *L. longiflorus* from *C. equisetifolia* host tree showed similar peak R_f values (0.23) in one carotenoid compound (peak no. 6 & 3, respectively). The leaf (X2) and bark (Y2) samples of *L. longiflorus* from *F. religiosa* host tree showed same peak R_f value (0.26) for one carotenoid compound (peak no. 4 & 3, respectively).

In general, the two carotenoid compounds (peak no. 4 & 6 of X1 and peak no. 1, 3 of X2) and two unknown compounds (peak no. 5 & 8 of X1 and peak no. 2 & 6 of X2) of the leaf samples of *L. longiflorus* collected from *C. equisetifolia* and *F. religiosa* host trees showed same peak R_f values (0.15, 0.19, 0.23 & 0.53, respectively) (Tab.-1). But, there is no similarities between the compounds of *L. longiflorus* bark samples collected from *C. equisetifolia* and *F. religiosa* host trees (Tab.-2).

Phytochemicals are reported to provide various biological functions leading to the promotion of health as well as the reduced risk of chronic diseases. Carotinoid (lutein) was found to scavenge SO, HO, NO radicals and inhibited *in vitro* lipid peroxidation. Its oral administration inhibited superoxide generation in macrophages *in vivo*. The oral administration of lutein in mice for one month significantly increased the activity of catalase, superoxide dismutase, glutathione reductase and glutathione in blood and liver while the activity of glutathione peroxidase and glutathione-S-transferase were found to be increased in the liver tissue [3]. Fat-soluble plant pigments, carotenoids, are extensively studied micronutrient phytochemicals for their potential health benefits. It is noteworthy that specific carotenoids may be responsible for different protective effects against certain diseases [4].

Several studies suggests that carotenoids (B-carotene) effectively increases intracellular oxidative stress by increasing ROS production, etc., in many tumour cells and this effect may be accompanied by anti-tumor activity; the carotenoid may also induce cell cycle arrest and apoptosis and, even, induced the loss of tumor cell viability [5-7]. The potential of antioxidant free radical scavenging activity of *L. longiflorus* leaf/bark samples obtained from *Casuarina equisetifolia* and *Ficus religiosa* host trees was reported [8, 9] and this may be due to the synchronous effect of carotenoids with other compounds. In this study, the HPTLC analysis of methanol extract of *L. longiflorus* leaf and bark samples from *C. equsetifolia* and *F. religiosa* host trees make certain the presence of carotenoids and the host trees showed impact on the nature and number of carotenoids present in the hemiparasitic plant.

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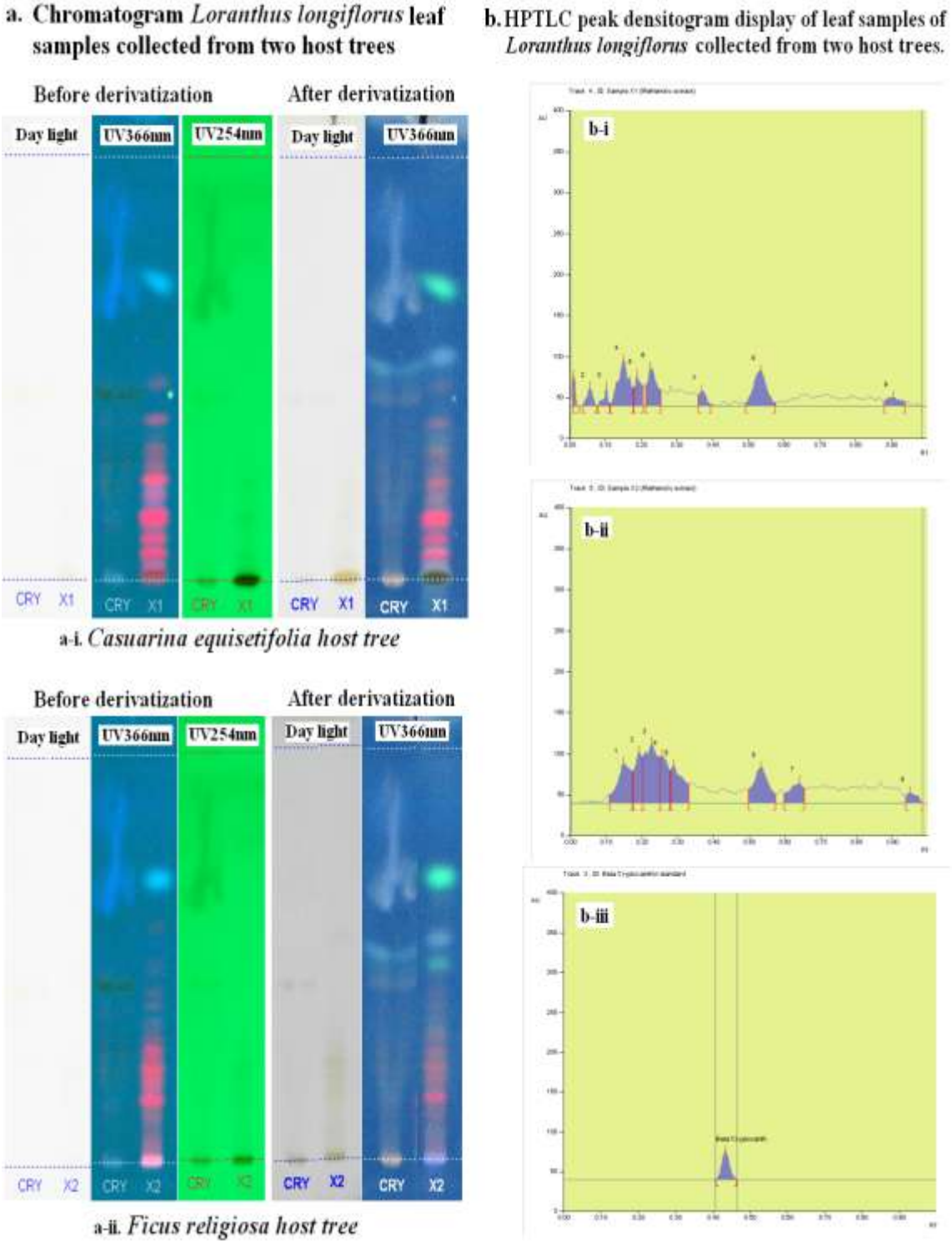

Figure 1: Chromatogram (a) and peak densitogram (b) shows carotenoids profile in the *Loranthus longiflorus* leaf samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; CRY- Cryptoxanthin standard -b-iii).

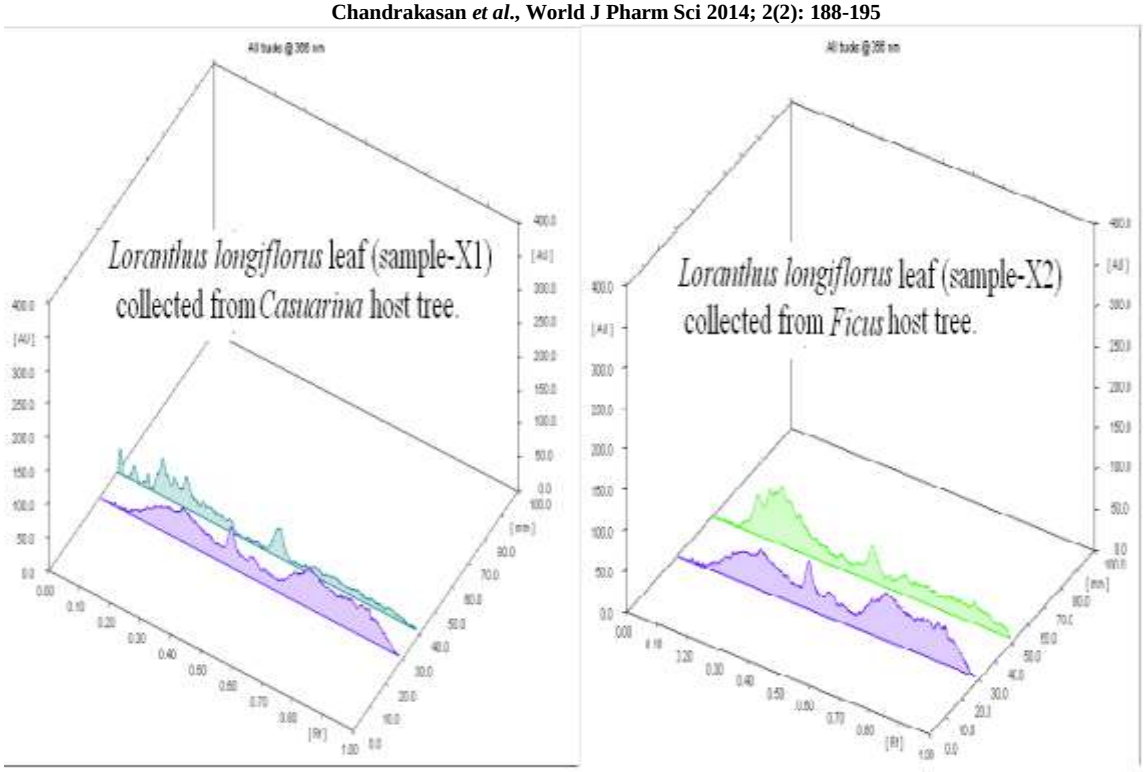

Figure 2: HPTLC-3D display of densitogram showing all tracks –plant samples (X1/ X2) and standard (Cryptoxanthin -blue coloured) scanned at 366nm.

Table 1: Peak table for HPTLC analysis of carotenoid 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	0.01	71.5	596.9	Unknown
X1	2	0.06	22.4	334.1	Carotenoid 1
X1	3	0.10	22.0	228.7	Carotenoid 2
X1	**4**	**0.15**	**55.8**	**1600.2**	**Carotenoid 3**
X1	**5**	**0.19**	**38.0**	**608.5**	**Unknown**
X1	**6**	**0.23**	**46.2**	**1127.0**	**Carotenoid 4**
X1	7	0.37	18.0	354.8	Carotenoid 5
X1	**8**	**0.53**	**43.2**	**1474.8**	**Unknown**
X1	9	0.91	10.5	377.1	Unknown
X2	**1**	**0.15**	**48.3**	**1682.6**	**Carotenoid 1**
X2	**2**	**0.19**	**62.2**	**1160.3**	**Unknown**
X2	**3**	**0.23**	**72.0**	**2424.9**	**Carotenoid 2**
X2	4	0.26	57.6	1175.4	Carotenoid 3
X2	5	0.29	45.9	1426.7	Unknown
X2	**6**	**0.53**	**43.4**	**1662.2**	**Unknown**
X2	7	0.64	25.5	879.1	Unknown
X2	8	0.95	12.9	328.8	Unknown
Control	**1**	**0.43**	**59.0**	**1772.1**	**Cryptoxanthin standard**

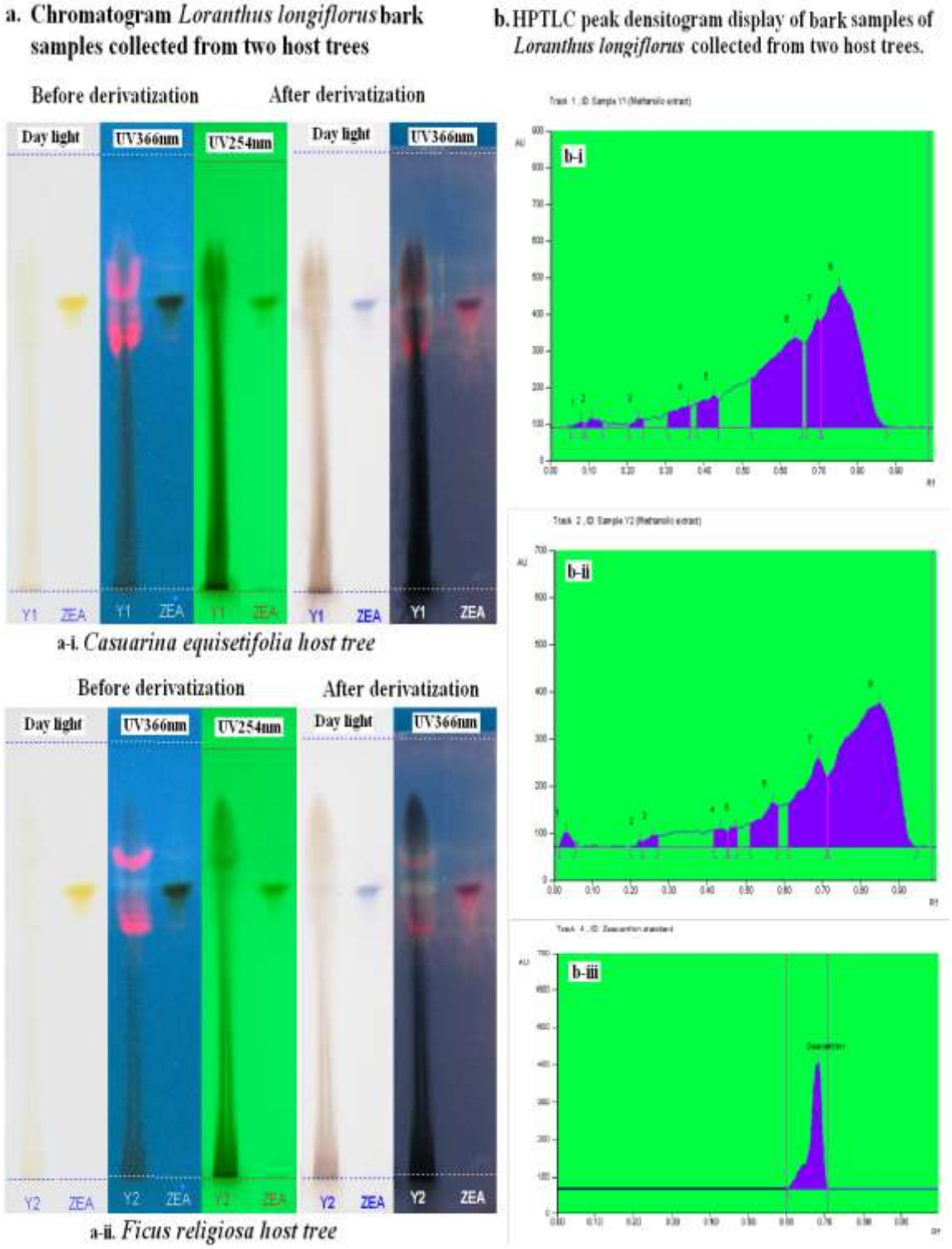

Figure 3: Chromatogram (a) and peak densitogram (b) shows carotenoids profile in the *Loranthus longiflorus* bark samples collected from *C. equisetifolia* (a-i/b-i) and *Ficus religiosa* (a-ii/b-ii) host trees (X1/X2-sample code; ZEA-Zeaxanthin standard -b-iii).

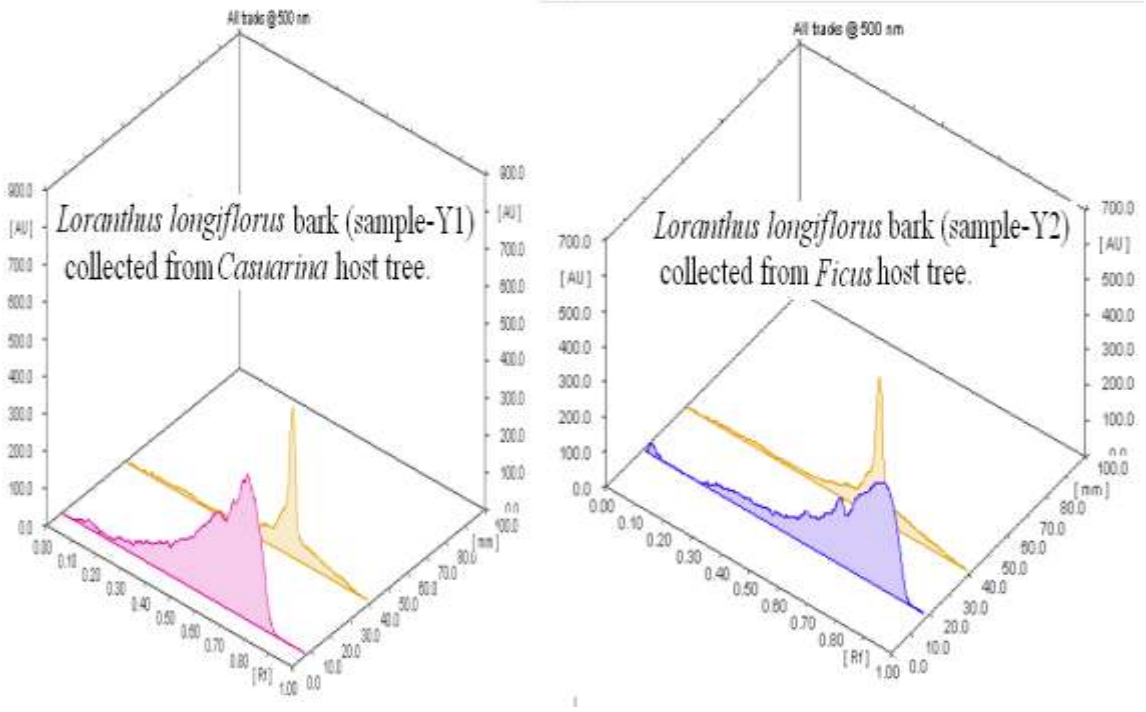

Figure 4: 3D display of densitogram showing all tracks –*Loranthus longiflorus* bark samples (Y1/ Y2) and standard (Zeaxanthin-Yellow coloured) scanned at 500nm.

Table 2: Peak table for HPTLC analysis of carotenoid 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	0.08	14.1	257.9	Unknown
Y1	2	0.11	24.5	694.1	Unknown
Y1	3	0.23	24.8	549.5	Carotenoid 1
Y1	4	0.36	56.6	2350.5	Unknown
Y1	5	0.43	86.7	3624.5	Unknown
Y1	6	0.64	243.9	21323.0	Carotenoid 2
Y1	7	0.70	296.7	8330.8	Carotenoid 3
Y1	8	0.75	385.9	31765.1	Unknown
Y2	1	0.03	30.7	665.4	Unknown
Y2	2	0.22	14.3	151.0	Unknown
Y2	3	0.26	24.9	642.6	Carotenoid 1
Y2	4	0.44	38.9	1088.4	Unknown
Y2	5	0.47	42.6	791.3	Unknown
Y2	6	0.57	93.1	4235.7	Carotenoid 2
Y2	7	0.69	187.0	11528.4	Carotenoid 3
Y2	8	0.85	304.3	38041.2	Unknown
Control	**1**	**0.68**	**400.2**	**15557.0**	**Zeaxanthin standard**

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