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ANALYSIS OF PHENYL PROPANOIDS PROFILE IN THREE *POLYGONUM* SPECIES BY HPTLC.

B. Ezhilan and R. Neelamegam*

Associate Professor, Department of Botany and Research Centre, S.T. Hindu College, Nagercoil -629 002, Kanyakumari (Dist.), Tamil Nadu, India.

***Correspondence for Author: R. Neelamegam**
Associate Professor, Department of Botany and Research Centre, S.T. Hindu College, Nagercoil -629 002, Kanyakumari (Dist.), Tamil Nadu, India.

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ABSTRACT

HPTLC analysis was carried out on phenyl propanoid compounds profile in the whole-plant samples of selected *Polygonum* species (*P. chinense, P. glabrum* and *P. barbatum*). The methanol extract of whole-plant samples obtained from *Polygonum* species (*P. chinense, P. glabrum* and *P. barbatum*) showed 9, 11 and 7 compounds, respectively, and were compared with eugenol standard. Among the compounds, 3 compounds in each sample were identified as phenyl propanoids while the others were unknown. One unknown and one phenyl propanoid compounds each from *P. chinense* and *P. glabrum* showing same peak R_f values (0.01/0.16, respectively). Similarly, another one unknown compound of *P. chinense* and *P. barbatum* also showed same peak R_f values (0.97, respectively), while all other detected compounds of *Polygonum* species showed no similarities in their peak R_f values.

KEYWORDS: HPTLC analysis, Phenyl propanoids, whole-plant material, Methanol extracts, *Polygonum* species.

INTRODUCTION

Phytochemicals are the natural bioactive compounds, found in plants, work with nutrients to form an integrated part of defense system against various diseases and stress conditions.[1] 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.[2] Multiple studies have proposed that phenyl propanoids can inhibit initiation of tumorigenesis or its development.[3] Last few years, much interest has been attracted to natural and synthetic phenyl propanoids for medicinal use as antioxidant, UV screens, anticancer, anti-virus, anti-inflammatory, wound healing, and antibacterial agents. They are of great interest for cosmetic and perfume industries as active natural ingredients.[4] The present study is aimed to list out the phenyl propanoid compound profile in the whole-plant samples of three *Polygonum* species –*P. chinense, P. glabrum and P. barbatum*.

MATERIALS AND METHODS

Study area

The test plant of three *Polygonum* species were collected during 2009 from Tirunelveli (*Polygonum chinense* Linn.) and Thoothukudi (*Polygonum glabrum* Willd. and *Polygonum barbatum* Linn.) districts of Tamil Nadu, India.

Polygonum species selected

The three species of *Polygonum* belongs to Polygonaceae were identified as *P. chinense, P. glabrum* and *P. barbatum* based on their morphological features and compared with plant characters described in the Flora of the Presidency of Madras[5], Indian Medicinal Plants[6] in order to confirm the species identification.

Preparation of whole plant dry powder of *Polygonum* species

The three *Polygonum* species were collected and dried separately at room temperature (30°C±2°C) for about two weeks to get a constant weight. The dried plant materials (as whole plant) were ground to powder by mechanical device and stored for further biochemical analysis.

Preparation of extract

The dried whole-plant materials of *Polygonum* samples (5g) from three species (*P. chinense, P. glabrum and P. barbatum*) were extracted separately with Methanol in Soxhlet apparatus for 3hrs. The extracts were cooled, filtered and concentrated using a vacuum flask evaporator. Finally these extracts were 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 *Polygonum* species (*P. chinense, P. glabrum*

and P. barbatum) were subjected to HPTLC analysis to assess the presence of various phenyl propanoid compounds.

HPTLC analysis for phenyl propanoid

- ***Test solution***: Methanol extracts of *P. chinense, P. glabrum* and *P. barbatum*.
- ***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.

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.

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.[7]

RESULTS AND DISCUSSION

The chromatogram (Figure: 1) shows phenyl propanoid profile of whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and is compared with eugenol 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 *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) (Figure:1).

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.

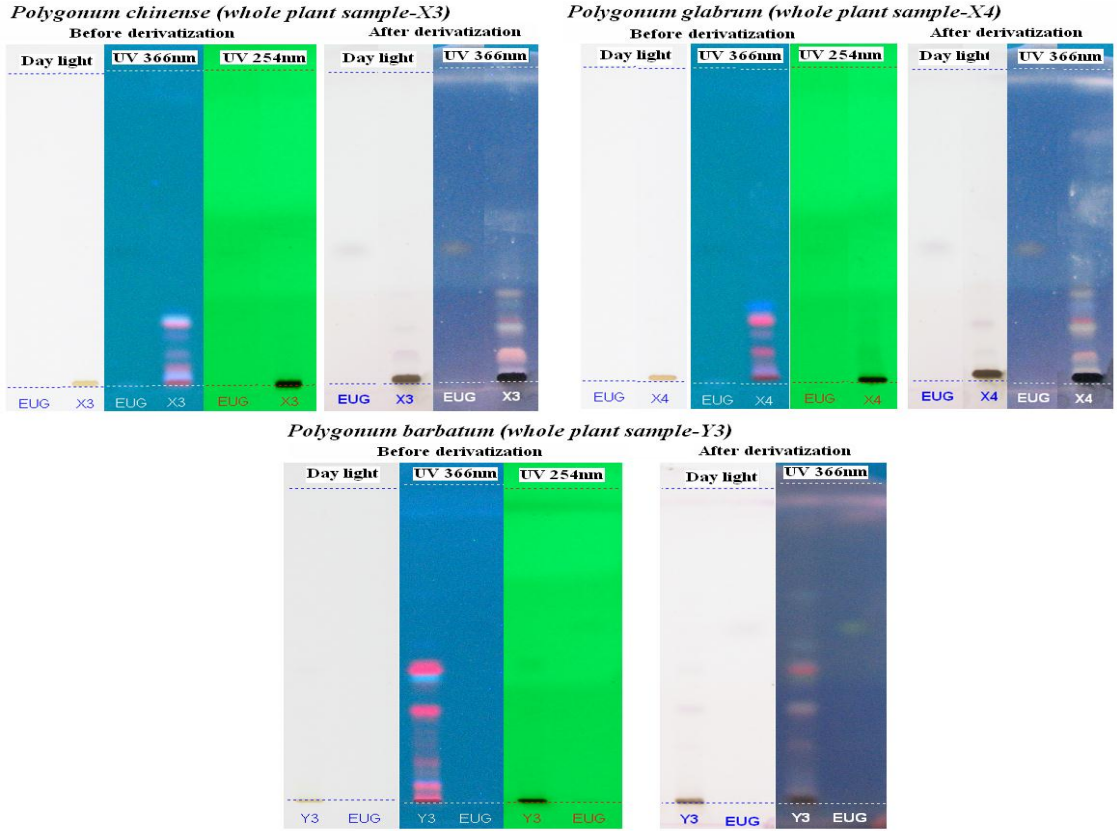

Figure 1: Chromatogram for phenyl propanoid compounds in the whole plant methanol extract of *Polygonum* species.

HPTLC analysis for phenyl propanoid profile in the whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) showed several peaks (R_f -values) of compounds (Table: 1; Figure:2) and were compared with eugenol standard. The densitogram (Figure: 2) shows the profile of phenyl propanoid compounds present in the whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3); and eugenol standard for samples scanned at 366nm.

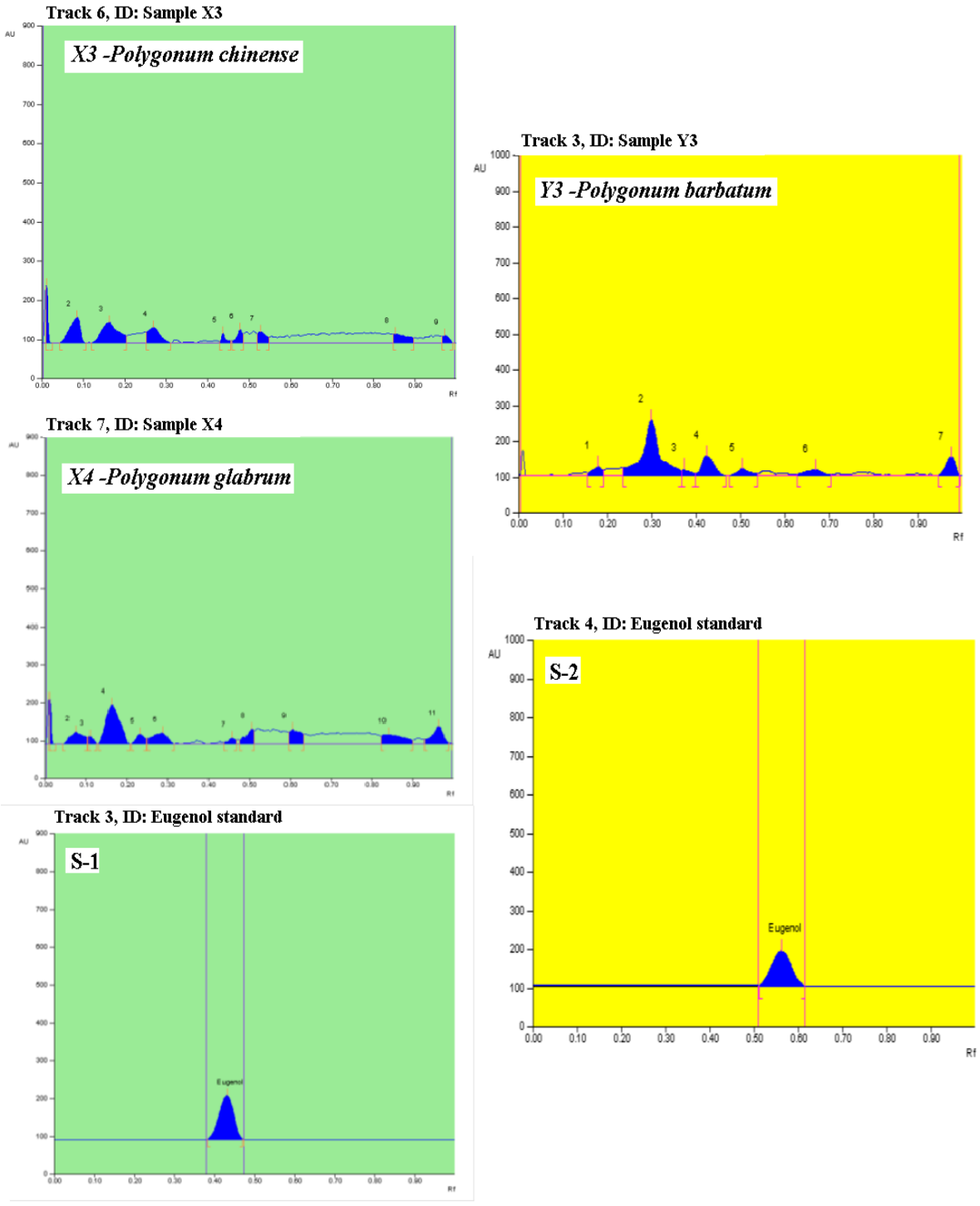

Figure 2: Densitogram showing the HPTLC analysis of phenyl propanoid compounds in the whole plant methanol extracts of *Polygonum* species (X3/X4/Y3); and Eugenol standard 'S-1' (for X3/X4) scanned at 366nm and Eugenol standard 'S-2' (for Y3) scanned at 500nm.

The 3D display of densitogram for phenyl propanoid profile shows all tracks of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and eugenol standard scanned at 366nm (for X3 & X4 samples) and 500nm (for Y3 sample) (Figure: 3).

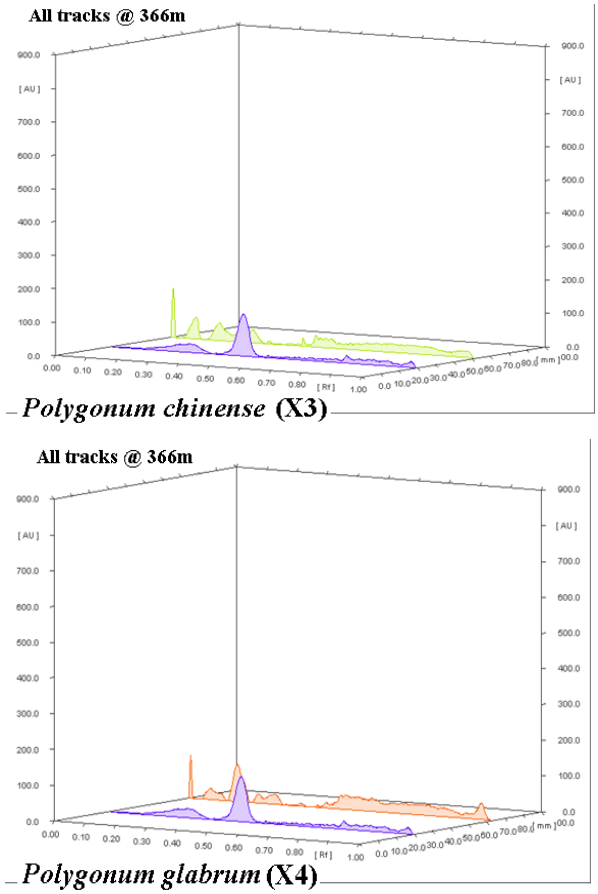

Figure 3: HPTLC densitogram 3D display of all tracks for phenyl propanoid compounds in the whole plant methanol extract of *Polygonum* species (X3/X4/Y3) and Standards (Eugenol for X3/X4/Y3).

The whole plant methanol extract of *P. chinense* (X3) showed 9 compounds with peak R_f values ranging from 0.01 to 0.97, peak height ranging from 17.9 to 146.3 and peak area ranging from 223.1 to 1946.2 as compared to eugenol standard (0.43, 124.3 and 4570.5, respectively). Among the 9 compounds detected, 3 were identified as phenyl propanoid compounds (peak no. 2-4) and the other compounds were unknown (Table: 1-X3; Figure: 2-X3)

Table 1: Peak table for HPTLC analysis of phenyl propanoid compound profile in the whole plant methanol extract of *Polygonum* species.

***P. chinense* (X3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X3	**1**	**0.01**	**146.3**	**642.9**	**Unknown**
X3	2	0.09	65.3	1516.7	Phenyl propanoid 1
X3	**3**	**0.16**	**53.1**	**1946.2**	**Phenyl propanoid 2**
X3	4	0.27	38.7	1053.6	Phenyl propanoid 3
X3	5	0.44	23.6	223.1	Unknown
X3	6	0.48	33.8	436.1	Unknown
X3	7	0.53	28.8	540.2	Unknown
X3	8	0.85	23.0	746.4	Unknown
X3	**9**	**0.97**	**17.9**	**271.8**	**Unknown**
***P. glabrum* (X4)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X4	**1**	**0.01**	**120.6**	**458.2**	**Unknown**
X4	2	0.07	32.1	963.7	Phenyl propanoid 1
X4	3	0.11	20.1	238.5	Unknown
X4	**4**	**0.16**	**103.4**	**3194.7**	**Phenyl propanoid 2**
X4	5	0.23	25.4	515.2	Unknown
X4	6	0.29	28.9	993.4	Phenyl propanoid 3
X4	7	0.46	16.1	258.2	Unknown
X4	8	0.51	39.1	728.6	Unknown
X4	9	0.60	38.3	1037.0	Unknown
X4	10	0.84	25.2	1213.9	Unknown
X4	11	0.96	46.5	1163.1	Unknown
***P. barbatum* (Y3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
Y3	1	0.18	24.0	504.4	Phenyl propanoid 1
Y3	2	0.30	156.3	5702.1	Phenyl propanoid 2
Y3	3	0.37	17.8	278.8	Unknown

Y3	4	0.42	54.3	1397.1	Phenyl propanoid 3
Y3	5	0.50	19.4	493.2	Unknown
Y3	6	0.67	15.8	591.8	Unknown
Y3	**7**	**0.97**	**52.2**	**1044.1**	**Unknown**
Control-1 (X3 & X4)	**1**	**0.43**	**124.3**	**4570.5**	**Eugenol standard**
Control-2 (Y3)	**1**	**0.56**	**99.2**	**4650.5**	**Eugenol standard**

Similarly, the whole plant methanol extract of *P. glabrum* (X4) showed 11 compounds with varied peak R_f values (0.01-0.96), peak height (16.1-120.6) and peak area (238.5-3194.7) as compared to eugenol standard (0.43, 124.3 and 4570.5, respectively). Out of 11 compounds detected, 3 compounds (peak No. 2, 4 & 6) were identified as phenyl propanoids and others were unknown (Table: 1-X4; Figure: 2-X4).

On the other hand, the whole plant methanol extract of *P. barbatum* (Y3) showed 7 compounds (Table: 1-Y3) with peak R_f values ranging from (0.18 to 0.97, peak height from 15.8 to 156.3 and peak area from 278.8 to 5702.1 as compared to eugenol standard (0.56, 99.2.3 and 4650.5, respectively) and out of 7 compounds, 3 were identified as phenyl propanoid compounds (peak No. 1, 2 & 4) and others were unknown (Table: 1-Y3; Figure: 2-Y3).

In general, the one unknown compound and one phenyl propanoid compound (peak No. 1 & 2) of *P. chinense* and of *P. glabrum* (peak No. 1 & 4) showed same peak R_f values (0.01 & 0.16, respectively). On the other hand, the one unknown compound of *P. chinense* (peak No. 9) and of *P. barbatum* (peak No. 7) show similar peak R_f values (0.97), while all other compounds of *Polygonum* species were differ from each other (Table: 1; Figure: 2).

The results of HPTLC analysis in the whole plant methanol extracts of *Polygonum* species reveals the presence of phenyl propanoid compounds and also indicate variations in the nature and number of phenyl propanoids present in the *Polygonum* species. Further, the HPTLC analysis on phenyl propanoid profile may help in the identification and evaluation of the quality of raw materials and the formulation of medicinal plants belongs to the family Polygonaceae.

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