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HPTLC ANALYSIS OF TANNIN COMPOUND PROFILE IN THREE *POLYGONUM* SPECIES

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ABSTRACT

HPTLC analysis was carried out on tannin 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 12, 10 and 15 compounds, respectively, and were compared with gallic acid standard. Among the compounds, 5, 5 and 2 compounds in each sample, respectively, were identified as tannins while the others were unknown. Three (One unknown and two tannin) compounds from each of *P. chinense* and of *P. glabrum* showed same peak R_f values (0.44, 0.65 and 0.98). Similarly, two unknown compounds of *P. chinense* and *P. barbatum* were also showed same peak R_f values (0.33 and 0.98). On the other hand, Three (two unknown and one tannin) compound of *P. glabrum* and of *P. barbatum* showed similar peak R_f values (0.06, 0.57 and 0.98), while all other compounds are differ from each other. The one unknown compound with R_f value 0.98 found in three *Polygonum* species tested. In general, the HPTLC analysis indicates the presence of various tannin compounds that differ in nature and number among the *Polygonum* species analyzed.

KEYWORDS

Polygonum species, HPTLC analysis, Tannin profile, Whole-plant samples and Methanol extracts.

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INTRODUCTON

Total tannins act as a good scavenger of free radicals either by donating hydrogen atom or by reducing them. This property is attributed by the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution and specific functional groups present in the tannins[1,2]. Tannins, the high molecular weight phenols, act as a good scavenger of free radicals either by donating hydrogen atom or by reducing them. Thus, the successive extracts may have more polyhydroxyl phenols, which may be acting synergistically with

other phytoconstituents to exhibit its antioxidant property as suggested by Thendral *et al*.[2]. *Polygonum* is a genus in the Polygonaceae family having many medicinal properties. In Chinese medicine, *Polygonum* extracts used to treat urinary infection[3]. Traditionally *Polygonum species* has been used in herbal medicine as a cure for digestive disorders and dandruff in Malaysia despite of its regular uses as food flavoring agent and appetizer in Malays cuisine; the essential oil extracted from *Polygonum* leaves is applied to hair to remove dandruff, used in aroma therapy[4] and in the perfume industry[5]. *Polygonum species* has also been reported to possess several pharmacological properties like antimicrobial activity[6], cytotoxic activity against HeLa (human cervical carcinoma)[7], antioxidant activity[8] and anticancer activity[9,10]. In the present study, it is aimed to estimate the tannin compound profile in the whole-plant samples of three *Polygonum* species -*P. chinense, P. glabrum* and *P. barbatum*.

MATERIAL 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[11], Indian Medicinal Plants[12] 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 tannin compounds.

HPTLC analysis for tannins

- **Test solution**: Methanol extracts of *P. chinense, P. glabrum* and *P. barbatum*.
- **Standard solution**: Methanol.
- **Standard chemical**: GA - Gallic acid was used as reference standard compound.
- **Mobile phase:** Toluene-ethyl acetate-formic acid-methanol (3: 3: 0.8: 0.2).
- **Spray reagent:** 5% Ferric chloride 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 UV 366nm 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[13].

RESULTS AND DISCUSSION

The chromatogram (Figure No.1) shows tannin profile of whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and is compared with gallic acid standard. Blue coloured zones at day light mode present in the gallic acid standard and plant samples track were observed in the chromatogram after derivatization and this confirmed the presence of tannin compounds in the *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) (Figure No.1).

The densitogram (Figure No.2) shows the profile of tannin compounds present in the methanolic extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3); and gallic acid standard scanned at UV 254 and 500nm.

The 3D display of densitogram for tannin profile shows all tracks of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and the gallic acid standard scanned at UV 254 and 500nm (Figure No.3).

HPTLC analysis for tannin 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 No.1, Figure No.2) and were compared with gallic acid standard.

The methanol extract of *P. chinense* (X3) showed 12 compounds with peak $R_f$values ranging from 0.02 to 0.98, peak height ranging from 22.6 to 238.8 and peak area ranging from 285.0 to 4892.1 as compared to gallic acid standard (0.45, 366.0 and 13180.3, respectively). Among the 12 compounds detected, 5 were identified as tannins (peak No. 5, 6, 8-10) and the others were unknown (Table No.1-X3; Figure No.2-X3).

Similarly, the *P. glabrum* (X4) whole plant methanol extract showed 10 compounds with varied peak R_f values (0.06-0.98), peak height (16.9-187.5) and peak area (219.4-7778.0) as compared to gallic acid standard (0.45, 366.0 and 13180.3, respectively). Out of 10 compounds detected, 5 compounds (peak No. 2, 4-7) were identified as tannin and others were unknown (Table No.1-X4; Figure No.2-X4).

On the other hand, the whole plant methanol extract of *P. barbatum* (Y3) showed 15 compounds (Table No.1-Y3) with peak R_f values ranging from (0.06 to 0.98, peak height from 19.9 to 168.8 and peak area from 155.8 to 8229.9 as compared to gallic acid standard (0.87, 149.7 and 6994.2, respectively) and out of 15 compounds detected, 2 were identified as tannins and others were unknown (Table No.1-Y3; Figure No.2-Y3).

In general, the one unknown compound and two tannin compounds (peak No. 12/5 and 8, respectively) of *P. chinense* and of *P. glabrum* (peak No. 10/5 and 7, respectively) showed same peak R_f values (0.98/0.44 and 0.65, respectively). Similarly two unknown compounds of *P. chinense* (peak No. 4 and 12) and *P. barbatum* (peak No. 6 and 15) were also showed same peak R_f values (0.33 and 0.98). On the other hand, one unknown compound (peak No. 1) and one tannin compound (peak No. 6) of *P. glabrum* and of *P. barbatum* (peak No. 1 and 10) showed similar peak R_f values (0.06 and 0.57, respectively), while all other compounds are differ from each other (Table No.1; Figure No.2).

Table No.1: Peak table for HPTLC analysis of tannin compound profile in the whole plant methanol extract of *Polygonum* species

S.No	***P. chinense* (X3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
1	X3	1	0.02	33.4	285.0	Unknown
2	X3	2	0.10	22.6	364.6	Unknown
3	X3	3	0.15	28.4	738.4	Unknown
4	X3	4	0.33	53.0	1681.4	Unknown
5	X3	5	0.44	143.8	4161.5	Tannin 1
6	X3	6	0.47	145.5	3223.1	Tannin 2
7	X3	7	0.58	58.7	1226.0	Unknown
8	X3	8	0.65	238.8	4892.1	Tannin 3
9	X3	9	0.70	59.7	1426.5	Tannin 4
10	X3	10	0.81	27.9	1270.7	Tannin 5
11	X3	11	0.84	27.0	615.0	Unknown
12	X3	12	0.98	28.9	470.0	Unknown
	***P. glabrum* (X4)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
13	X4	1	0.06	16.9	219.4	Unknown
14	X4	2	0.11	107.6	2842.3	Tannin 1
15	X4	3	0.25	39.6	1100.0	Unknown
16	X4	4	0.32	89.6	2817.9	Tannin 2
17	X4	5	0.44	180.7	7778.0	Tannin 3
18	X4	6	0.57	187.5	4184.8	Tannin 4
19	X4	7	0.65	174.0	6362.0	Tannin 5
20	X4	8	0.77	119.2	6792.5	Unknown
21	X4	9	0.82	108.7	3236.3	Unknown
22	X4	10	0.98	29.7	392.2	Unknown
	***P. barbatum* (Y3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
23	Y3	1	0.06	27.9	440.5	Unknown
24	Y3	2	0.07	33.5	384.4	Unknown
25	Y3	3	0.14	68.6	1945.8	Unknown
26	Y3	4	0.23	163.2	8229.9	Unknown
27	Y3	5	0.29	165.4	4493.4	Unknown
28	Y3	6	0.33	168.8	6215.3	Unknown
29	Y3	7	0.41	158.3	6638.2	Unknown
30	Y3	8	0.49	127.7	3351.5	Unknown
31	Y3	9	0.55	116.2	3103.3	Unknown
32	Y3	10	0.57	115.6	2104.4	Tannin 1
33	Y3	11	0.76	100.0	1780.1	Unknown
34	Y3	12	0.87	87.4	1379.7	Tannin 2
35	Y3	13	0.89	77.0	1633.9	Unknown
36	Y3	14	0.94	140.7	3663.0	Unknown
37	Y3	15	0.98	19.9	155.8	Unknown
38	**Control-1 (X3 and X4)**	1	0.45	366.0	13180.3	Gallic acid standard
39	**Control-2 (Y3)**	1	0.87	149.7	6994.2	Gallic acid standard

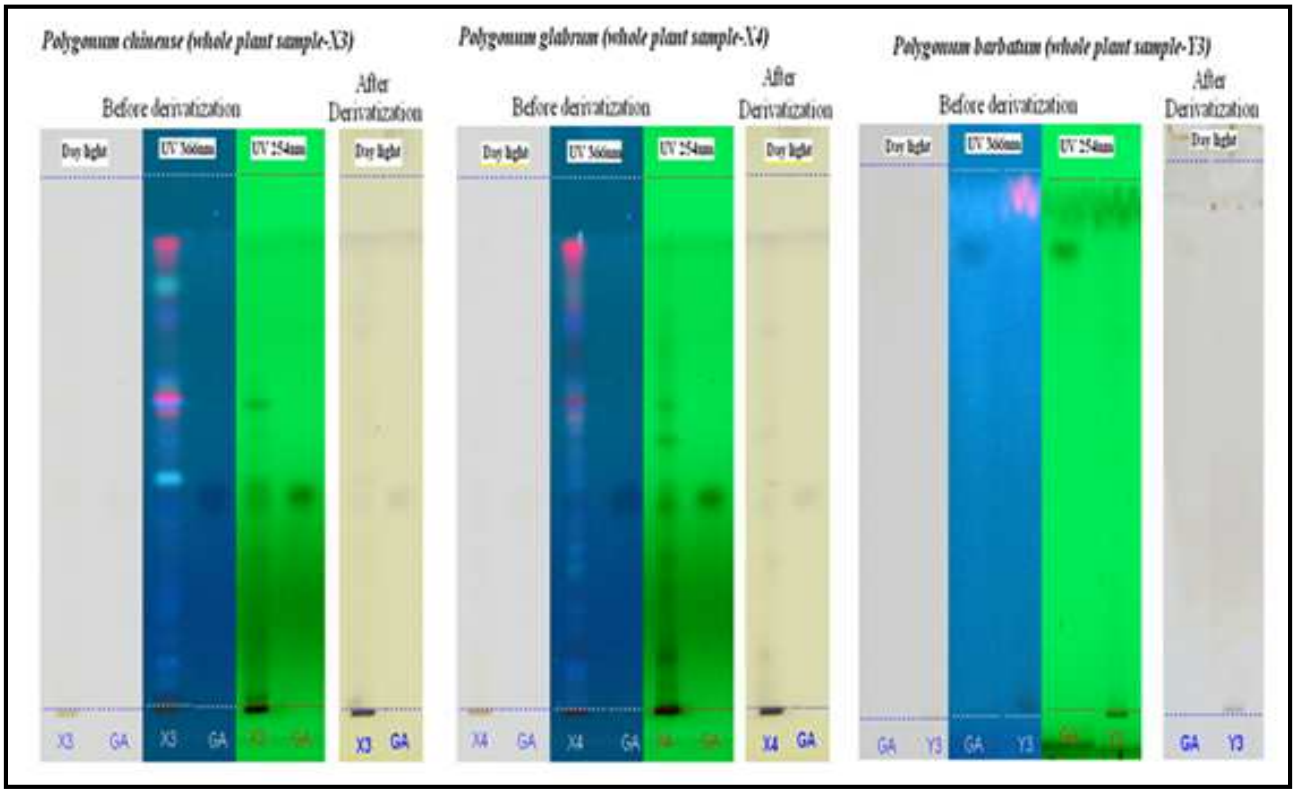

Figure No.1: Chromatogram for tannin compounds in the whole plant methanol extract of *Polygonum* species

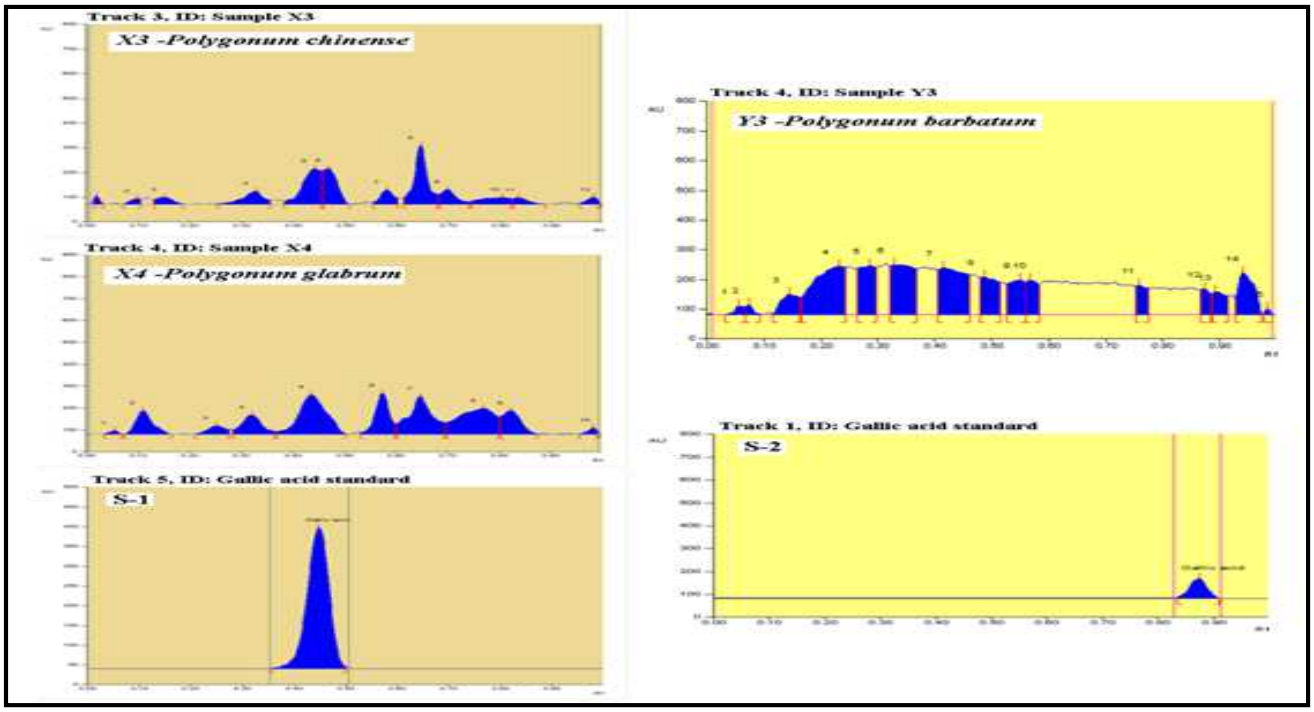

Figure No.2: Densitogram showing the HPTLC analysis of tannin compounds in the whole plant methanol extracts of *Polygonum* species (X3/X4/Y3); and Gallic acid standard 'S-1' (for X3/X4) scanned at 254nm and Gallic acid standard 'S-2' (for Y3) scanned at 500nm

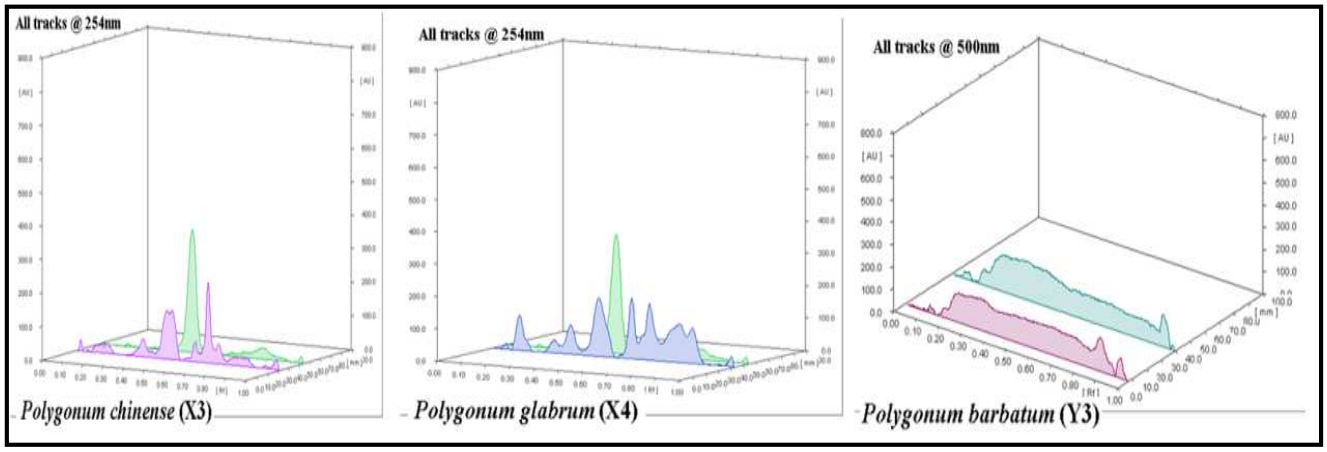

Figure No.3: HPTLC densitogram 3D display of all tracks for tannin compounds in the whole plant methanol extract of *Polygonum* species (X3/X4/Y3) and Standards (Gallic acid for X3/X4/Y3)

CONCLUSION

The results of present study indicate that the HPTLC analysis of the whole plant methanol extracts of three *Polygonum* species make certain the presence of tannin compounds and the nature and number of tannins present in the *Polygonum* species is varied. The tannin compounds detected in the methanol extract of three *Polygonum* species may play an important role in the identification and evaluation of the raw materials quality and formulations this medicinally important *Polygonum* species.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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