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

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

Correspondence
R Neelamegam
Associate Professor
Department of Botany and Research Centre, S.T. Hindu College, Nagercoil, Kanyakumari (Dist.), Tamil Nadu, India

Determination of steroid compounds profile in three *Polygonum* species by HPTLC

B Ezhilan and R Neelamegam

Abstract
HPTLC analysis was carried out to determine the steroid 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 6, 6 and 5 compounds, respectively, and were compared with stigmasterol and solasodine standards. Among the compounds, 4, 3 and 2 compounds in each sample, respectively, was identified as steroids while the others were unknown. Two steroid compounds each from *P. chinense* and *P. glabrum* showing same peak R_f values (0.69/0.89, respectively). Similarly, one unknown compound each from *P. glabrum* and *P. barbatum* also showed same peak R_f values (0.14), while all other compounds of *Polygonum* species showed no similarities in their peak R_f values of compounds detected. The HPTLC analysis of whole plant methanol extracts of *Polygonum* species shows variations in the nature and number of steroids from one another.

Keywords: *Polygonum* species, whole-plant material, methanol extracts, HPTLC analysis, steroids profile

1. Introduction
Plants have had a major impact on modern medicine in providing important steroids. Plant steroids are types of natural organic compounds found in plants. Till today, more than four thousand plant species have been investigated which has resulted in the identification of some thirty naturally occurring steroids sapogenins many of which could provide valuable source materials for steroids compounds. Many types of plant steroids exist and play important roles in the biological processes of plants, such as growth and development, cell division, and resistance to damage from environmental stresses like cold weather. There are many steroids and sterols that are important in health and medicine, and some that may be used as medications. *Polygonum* is a genus in the Polygonaceae family having many medicinal properties. In Chinese medicine, *Polygonum* extracts used to treat urinary infection [1]. 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 [2] and in the perfume industry [3]. *Polygonum species* has also been reported to possess several pharmacological properties like antimicrobial activity [4], cytotoxic activity against HeLa (human cervical carcinoma) [5], antioxidant activity [6] and anticancer activity [7, 8]. In the present study, it is aimed to estimate the steroid compounds profile in the whole-plant samples of three *Polygonum* species –*P. chinense, P. glabrum* and *P. barbatum*.

2. Materials and Methods
2.1 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.

2.2 *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 [9] (Gamble, 1956),

Indian Medicinal Plants [10] (Kirtikar and Basu, 2003) in order to confirm the species identification.

2.3 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.

2.4 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.

2.5 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 steroid compounds.

2.6 HPTLC analysis for steroid

- ***Test solution***: Methanol extracts of *P. chinense, P. glabrum* and *P. barbatum*.
- ***Standard solution***: Methanol.
- ***Standard chemical***: SGL – Stigmasterol and solasodine were used as reference standard compounds.
- ***Mobile phase***: Chloroform-Acetone (8: 2).
- ***Spray reagent***: Anisaldehyde sulphuric acid reagent.

2.7 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.

2.8 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.

2.9 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.

2.10 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.

2.11 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. [11]

3. Results and Discussion

The results of HPTLC analysis in the whole-plant samples of selected *Polygonum* species are presented in Table 1 and Figures 1 to 3. The chromatogram (Figure 1) shows steroid profile of whole plant methanolic extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* – Y3) and is compared with stigmasterol/solasodine standards. Blue, blue-violet coloured zones at day light and UV 366nm mode present in the stigmasterol/solasodine standards and plant samples tracks were observed in the chromatogram after derivatization and this confirmed the presence of steroid compounds in the *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) (Figure 1).

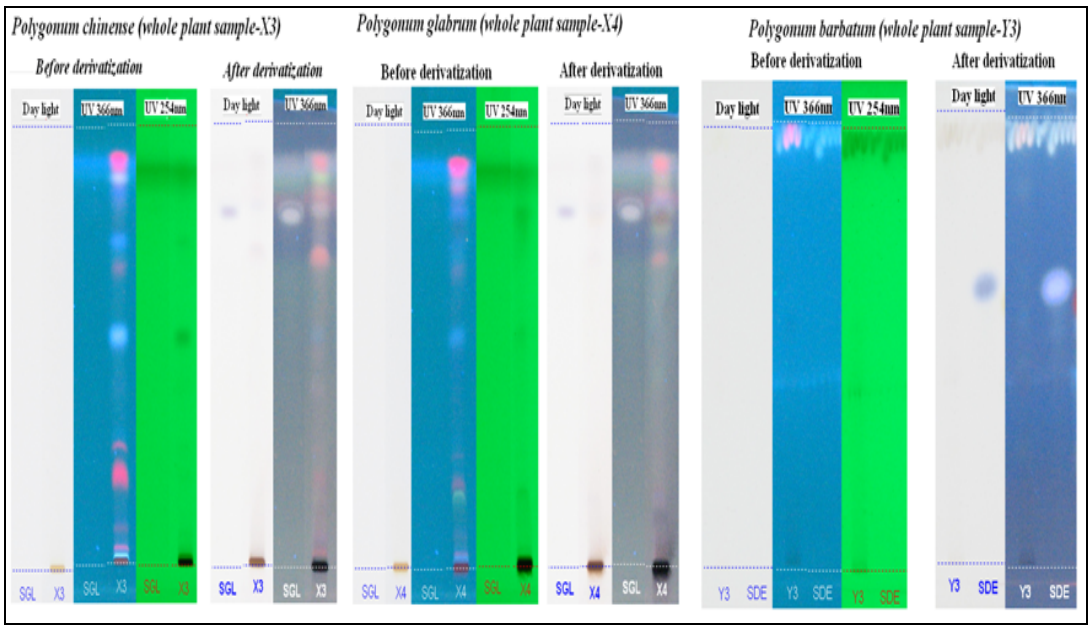

Fig 1: Chromatogram for steroid compounds in the whole plant methanol extract of *Polygonum* species.

The densitogram (Figure 2) shows the profile of steroid compounds present in the whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3); and stigmasterol standard for X3 & X4 samples and solasodine standard for Y3 sample scanned at 366nm and 500nm, respectively.

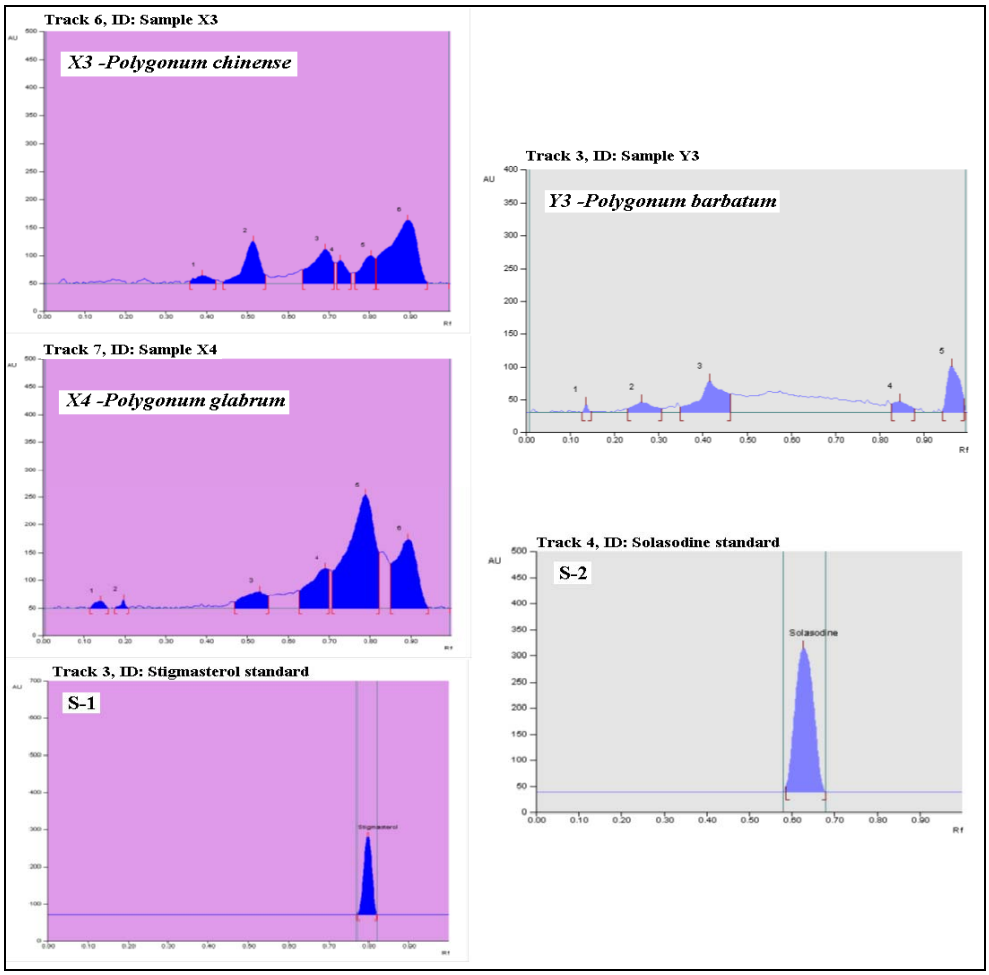

Fig 2: Densitogram showing the HPTLC analysis of steroid compounds in the whole plant methanol extracts of *Polygonum* species (X3/X4/Y3); and Stigmasterol standard 'S-1' (for X3/X4) scanned at 366nm and Solasodine standard 'S-2' (for Y3) scanned at 500nm.

The 3D display of densitogram for glycoside profile shows all tracks of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and stigmasterol/solasodine standards scanned at 366nm and 500nm, respectively (Figure 3).

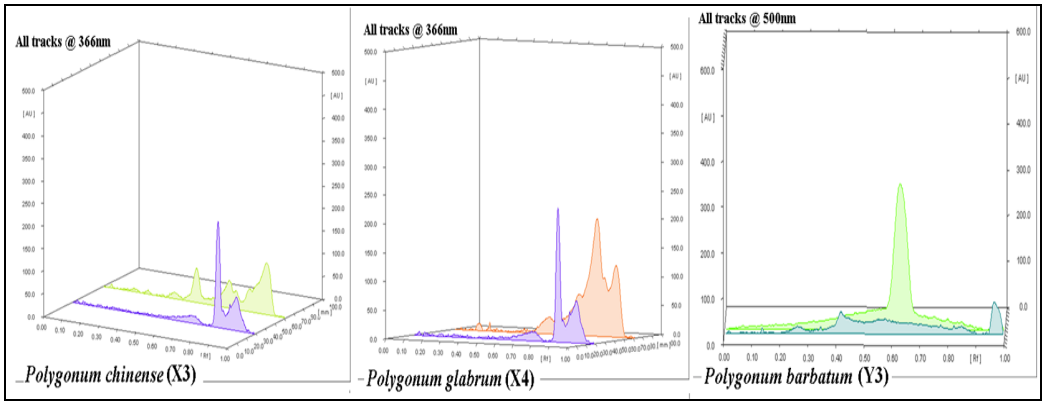

Fig 3: HPTLC densitogram 3D display of all tracks for steroid compounds in the whole plant methanol extract of *Polygonum* species (X3/X4/Y3) and Standards (Stigmasterol for X3/X4 and Solasodine for Y3).

HPTLC analysis for steroid 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 stigmasterol/solasodine standards.

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

***P. chinense* (X3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X3	1	0.39	14.6	516.5	Unknown
X3	2	0.51	75.5	2704.9	Unknown
X3	3	0.69	61.3	2731.9	Steroid 1
X3	4	0.73	41.0	927.8	Steroid 2
X3	5	0.80	50.2	1564.8	Steroid 3
X3	6	0.89	113.4	7064.2	Steroid 4
P. glabrum (X4)	Peak	Rf	Height	Area	Assigned substance
X4	1	0.14	12.8	293.6	Unknown
X4	2	0.20	15.2	143.9	Unknown
X4	3	0.53	30.0	1529.6	Unknown
X4	4	0.69	71.5	3169.4	Steroid 1
X4	5	0.79	204.1	12336.6	Steroid 2
X4	6	0.89	124.4	5902.8	Steroid 3
P. barbatum (Y3)	Peak	Rf	Height	Area	Assigned substance
Y3	1	0.14	11.8	84.5	Unknown
Y3	2	0.26	15.2	632.6	Steroid 1
Y3	3	0.41	47.4	2354.5	Steroid 2
Y3	4	0.84	17.1	533.2	Unknown
Y3	5	0.96	70.3	1812.8	Unknown
Control-1 (X3 & X4)	1	0.80	232.4	4871.6	Stigmasterol standard
Control-2 (Y3)	1	0.63	318.8	15382.6	Solasodine standard

Polygonum chinense (X3) whole plant methanol extract showed 6 compounds with peak R_f values ranging from 0.39 to 0.89, peak height ranging from 14.6 to 113.4 and peak area ranging from 516.5 to 7064.2 as compared to stigmasterol standard (0.80, 232.4 and 4871.6, respectively). Among the 6 compounds detected, 4 were identified as steroids (peak No. 3-6) and others were unknown (Table 1-X3; Figure 2-X3).

The whole plant methanol extract of *P. glabrum* (X4) showed 6 compounds with varied peak R_f values (0.14-0.89), peak height (12.8-204.1) and peak area (143.9-12336.6) as compared to solasodine standard (0.80, 232.4 and 4871.6, respectively). Out of 6 compounds detected, 3 compounds (peak No. 4-6) were identified as steroid and others were unknown (Table 1-X4; Figure 2-X4).

On the other hand, the whole plant methanol extract of *P. barbatum* (Y3) showed 5 compounds (Table 1-Y3) with peak R_f values ranging from (0.14 to 0.96, peak height from 11.8 to 70.3 and peak area from 84.5 to 2354.5 as compared to stigmasterol standard (0.63, 318.8 and 15382.6, respectively) and out of 5 compounds detected, 2 were identified as steroids (peak No. 2 & 3) and others were unknown (Table 1-Y3; Figure 2-Y3).

In general, the two steroid compounds (peak No. 3 & 6) of *P. chinense* and of *P. glabrum* (peak No. 4 & 6) showed same peak R_f values (0.69 & 0.89, respectively). On the other hand, one unknown compound (peak No. 1) of *P. glabrum* and *P. barbatum* showed similar peak R_f values (0.02 & 0.97), respectively (Table 1; Figure 2), while there is no similarities between the compounds detected in the whole plant methanol extracts of *P. chinense* and *P. barbatum*.

4. Conclusion

The results of present study indicate that the HPTLC analysis of methanol extracts of *Polygonum* species make certain the presence of steroid compounds and the nature and number of steroids present in the *Polygonum* species is differ from one another. The steroid 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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