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COMPARATIVE HPTLC EVALUATION FOR GLYCOSIDE COMPOUND PROFILE IN THREE *POLYGONUM* SPECIES

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Keywords:

HPTLC analysis, Glycosides, whole-plant materials, methanol extracts, *Polygonum* species

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ABSTRACT

HPTLC analysis was carried out to compare the glycoside 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 11, 9 and 11 compounds, respectively, and were compared with stevioside standard. Among the compounds, 6, 4 and 3 compounds in each sample, respectively, was identified as glycosides while the others were unknown. One unknown and one glycoside compounds each from *P. chinense* and *P. glabrum* showing same peak R_f values (0.28/0.85, respectively). Similarly, another one unknown compound and two glycoside compounds of *P. chinense* and *P. barbatum* also showed same peak R_f values (0.01, 0.14 & 0.78, respectively), while all other compounds of *Polygonum* species showed no similarities in their peak R_f values of compounds detected.

INTRODUCTION

The pharmacological action of crude drug is determined by the nature of its constituents. There is a need to evaluate these crude drugs on modern scientific lines such as phytochemical analysis using HPTLC.[1] Glycosides one of the secondary metabolites, are widely used in pharmaceutical industries in the production of steroidal hormones, vitamins, active pharmaceutical ingredients and also for therapeutic purposes such as congestive heart failure (digitalis), cardiotonic and diuretic, rodenticide, anticancer, laxative and purgative, anti-inflammatory, emulsifier, expectorant, liver disorders, etc. *Polygonum* is a genus in the Polygonaceae family having many medicinal properties. In Chinese medicine, a *Polygonum* extracts used to treat urinary infection.[2] 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[3] and in the perfume industry.[4] ***Polygonum* species** has also been reported to possess several pharmacological properties like antimicrobial activity,[5] cytotoxic activity against HeLa (human cervical carcinoma),[6] antioxidant activity[7] and anticancer activity.[8, 9] In the present study, it is aimed to estimate the glycoside 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,[10] Indian Medicinal Plants[11] 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 glycoside compounds.

HPTLC analysis for glycosides:

- ***Test solution***: Methanol extracts of *P. chinense*, *P. glabrum* and *P. barbatum*.
- ***Standard solution***: Methanol.
- ***Standard chemical***: STE – Stevioside was used as reference standard compound.
- ***Mobile phase***: Ethyl acetate-Ethanol-Water (8: 2: 1.2).
- ***Spray reagent***: Chloramine-T 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 by 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 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.[12]

RESULTS and DISCUSSION

The chromatogram (Figure 1) shows glycoside profile of whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and is compared with stevioside standard. Blue-yellow coloured fluorescent zones present in the stevioside standard and plant samples tracks at UV 366nm mode were observed in the chromatogram after derivatization and this confirmed the presence of glycoside compounds

may be in the *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) (Figure 1). HPTLC analysis for glycoside 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 stevioside standard. The densitogram (Figure 2) showed the profile of glycoside compounds present in the whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3); and stevioside standard for samples scanned at 366nm and 500nm.

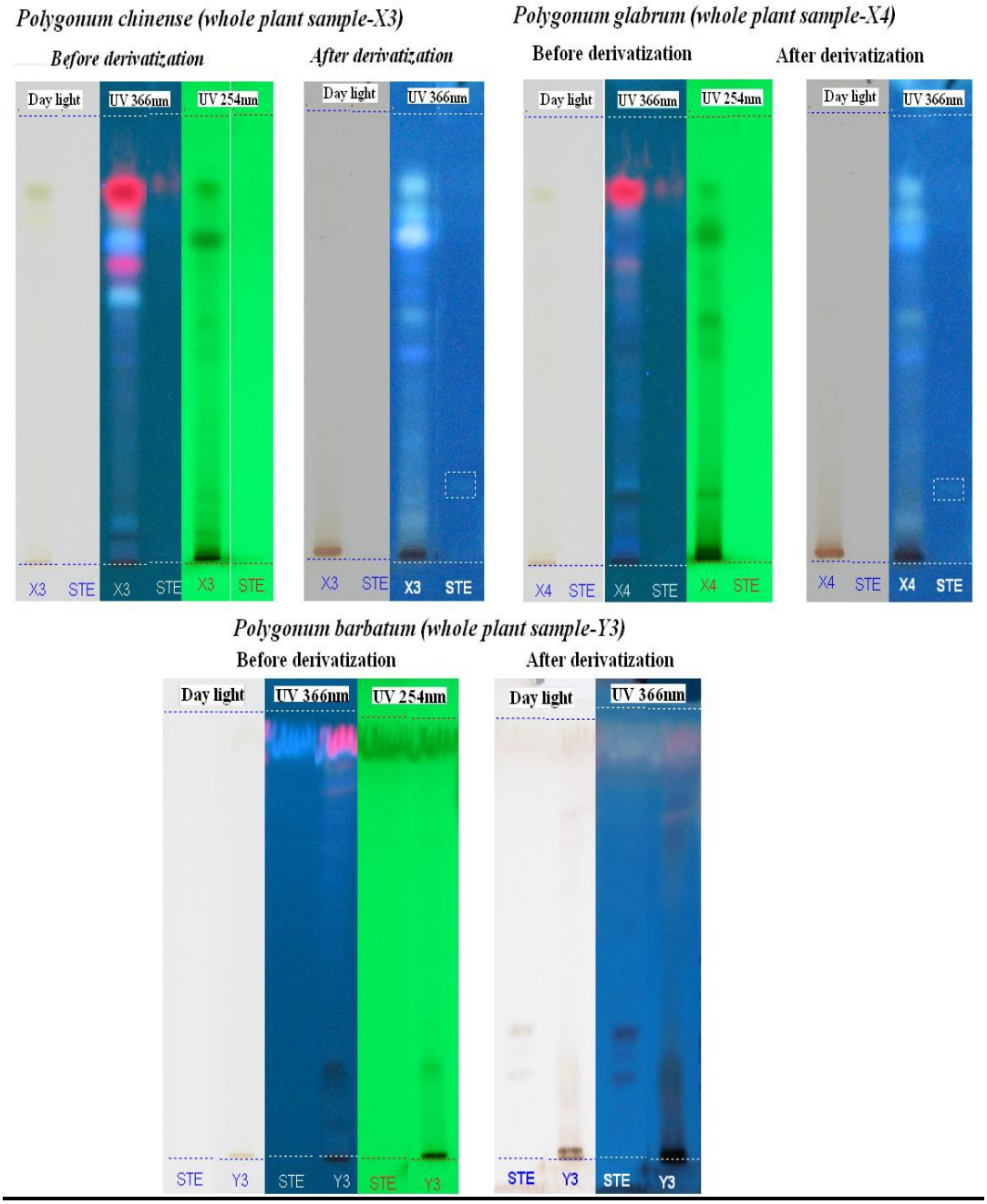

Figure 1: Chromatogram for glycoside compounds in the whole plant methanol extract of *Polygonum* species.

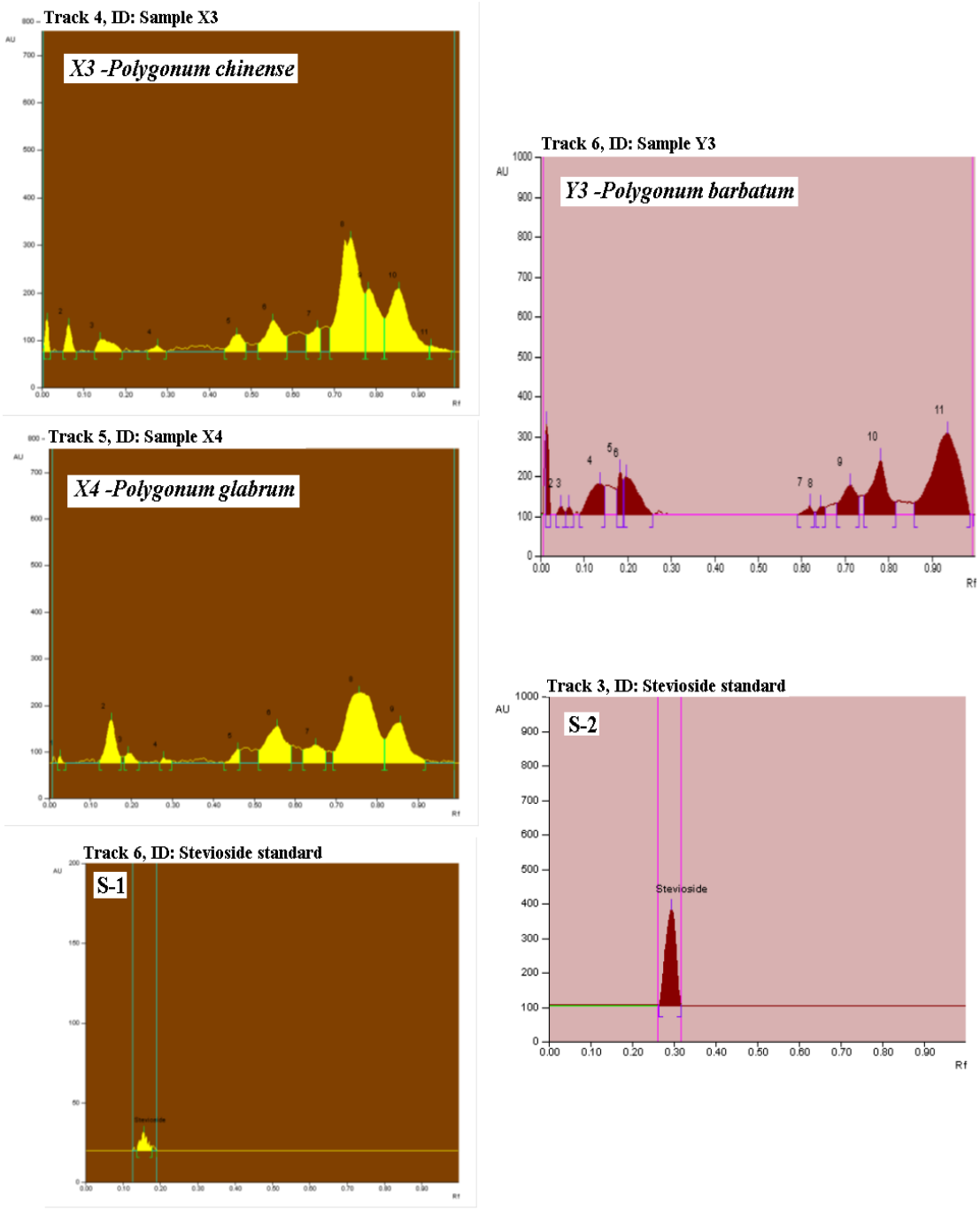

Figure 2: Densitogram showing the HPTLC analysis of glycoside compounds in the whole plant methanol extracts of *Polygonum* species (X3/X4/Y3); and Stevioside standard 'S-1' (for X3/X4) scanned at 366nm and Stevioside standard 'S-2' (for Y3) scanned at 500nm.

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 stevioside standard scanned at 366nm and 500nm (Figure 3).

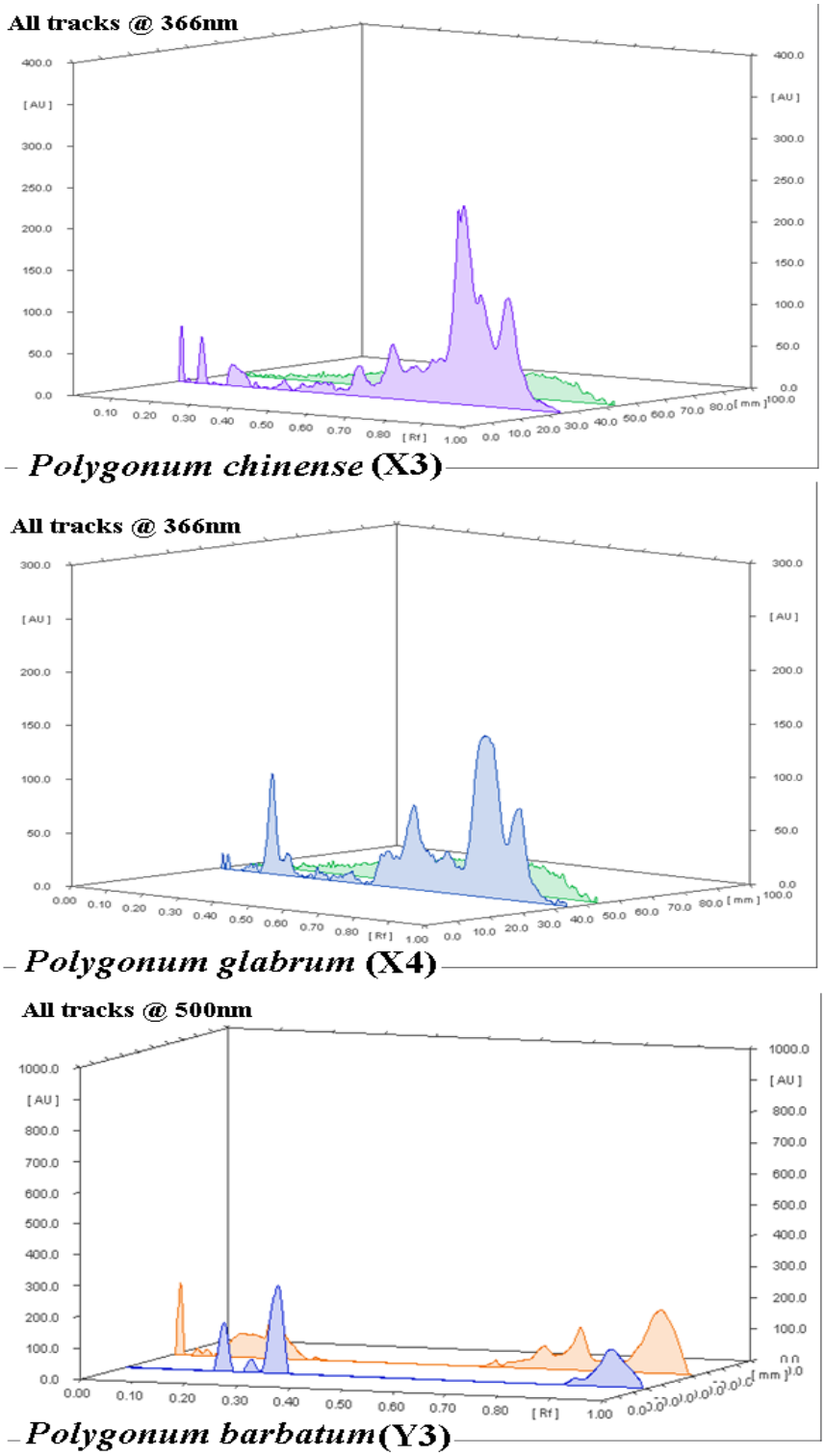

Figure 3: HPTLC densitogram 3D display of all tracks for glycoside compounds in the whole plant methanol extract of *Polygonum* species (X3/ X4/ Y3) and Standards (SteviosideX3/X4/Y3).

The whole plant methanol extract of *P. chinense* (X3) showed 11 compounds with peak R_f values ranging from 0.01 to 0.93, peak height ranging from 12.6 to 39.6 and peak area

ranging from 223.1 to 10640.7 as compared to stevioside standard (0.16, 11.7 and 295.5, respectively). Among the 11 compounds detected, 7 were identified as glycosides (peak no. 3, 5-10) and the others were unknown (Table 1-X3; Figure 2-X3).

But, the *P. glabrum* (X4) whole plant methanol extract showed 9 compounds with varied peak R_f values (0.03-0.85), peak height (10.8-150.2) and peak area (93.4-10197.4) as compared to stevioside standard (0.16, 11.7 and 295.5, respectively). Out of 9 compounds detected, 4 compounds (peak No. 5, 6, 8 & 9) were identified as glycosides and others were unknown (Table 1-X4; Figure 2-X4).

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

***P. chinense* (X3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X3	**1**	**0.01**	**66.8**	**475.4**	**Unknown**
X3	2	0.06	56.5	658.8	Unknown
X3	**3**	**0.14**	**25.9**	**860.1**	**Unknown**
X3	**4**	**0.28**	**12.6**	**223.1**	**Unknown**
X3	5	0.47	36.1	1024.7	Glycoside 1
X3	6	0.55	65.8	2370.8	Glycoside 2
X3	7	0.66	51.0	1201.8	Glycoside 3
X3	8	0.74	239.6	10640.7	Glycoside 4
X3	**9**	**0.78**	**132.6**	**3877.6**	**Glycoside 5**
X3	**10**	**0.85**	**132.2**	**5955.2**	**Glycoside 6**
X3	11	0.93	12.8	291.1	Unknown
***P. glabrum* (X4)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X4	1	0.03	14.2	93.4	Unknown
X4	2	0.15	93.2	1919.7	Unknown
X4	3	0.19	21.0	409.0	Unknown
X4	**4**	**0.28**	**10.8**	**151.7**	**Unknown**
X4	5	0.46	29.8	465.8	Glycoside 1
X4	6	0.56	78.6	3466.0	Glycoside 2
X4	7	0.65	39.1	1527.0	Unknown
X4	8	0.76	150.2	10197.4	Glycoside 3
X4	**9**	**0.85**	**86.3**	**3820.3**	**Glycoside 4**
***P. barbatum* (Y3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
Y3	**1**	**0.01**	**227.3**	**1351.6**	**Unknown**
Y3	2	0.05	20.6	217.3	Unknown
Y3	3	0.07	18.8	176.6	Unknown
Y3	**4**	**0.14**	**75.3**	**2397.3**	**Glycoside 1**
Y3	5	0.18	108.8	1230.5	Unknown
Y3	6	0.20	94.0	2758.2	Unknown
Y3	7	0.62	22.2	295.0	Unknown
Y3	8	0.64	19.1	285.0	Unknown
Y3	9	0.71	73.4	2235.7	Glycoside 2
Y3	**10**	**0.78**	**137.5**	**4393.6**	**Glycoside 3**
Y3	11	0.94	203.3	11727.3	Unknown
Control-1 (X3 & X4)	**1**	**0.16**	**11.7**	**295.5**	**Stevioside standard**
Control-2 (Y3)	**1**	**0.29**	**282.0**	**6513.8**	**Stevioside standard**

On the other hand, the whole plant methanol extract of *P. barbatum* (Y3) showed 11 compounds (Tab. 1-Y3) with peak R_f values ranging from (0.01 to 0.94, peak height from 18.8 to 227.3 and peak area from 176.6 to 11727.3 as compared to stevioside standard (0.29, 282.0, and 6513.8 respectively) and out of 11 compounds, 3 were identified as glycosides (peak No. 4, 9 & 10) and others were unknown (Table 1-Y3; Figure 2-Y3).

In general, one unknown and one glycoside compounds (peak No. 4/10, respectively) of *P. chinense* and of *P. glabrum* (peak No. 4/9, respectively) showed same peak R_f values (0.28/0.85, respectively). Similarly, another one unknown compound and two glycoside compounds (peak No. 1/3 & 9) of *P. chinense* and of *P. barbatum* (peak No. 1/4 &10) also showed same peak R_f values (0.01/0.14 & 0.78, respectively), while all other compounds of *Polygonum* species showed no similarities in their peak R_f values of compounds detected (Table 1; Figure 2).

The results of present study indicate that the HPTLC analysis of methanol extracts of *Polygonum* species not only make certain the presence of glycosides and also reveals the variations in the nature and number of glycosides present in the *Polygonum* species. The glycosides detected in the methanol extract of *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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