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Research Article

HPTLC Analysis of Flavonoid Compound Profile in the whole-plant Methanol Extract of *Polygonum* Species.

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Abstract

A comparison of HPTLC analysis was carried out to record the flavonoid compounds profile in the whole-plant samples of three *Polygonum* species (*P. chinense, P. glabrum* and *P. barbatum*). The methanol extract of whole-plant samples obtained from *P. chinense, P. glabrum* and *P. barbatum* shows 8, 6 and 10 compounds, respectively, and were compared with rutin and apigenin standards. Among the compounds, 2 compounds in each sample were identified as flavonoids while the others were unknown. One flavonoid compound from each of *P. chinense* and of *P. barbatum* showed same peak R_f values (0.38). Similarly, one unknown compound from *P. glabrum* and *P. barbatum* were also showed same peak R_f values (0.97) while all other compounds are differ from each other. All the three *Polygonum* species tested differ in their nature and number of flavonoid compounds detected.

Keywords: Flavonoids, HPTLC, Methanol extracts, *Polygonum chinense, Polygonum glabrum, Polygonum barbatum*.

Introduction

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer and Jeremy, 2008) according to chemical structure flavonoids are categorized, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Flavonoids have aroused considerable research interest recently because of their potential beneficial effects on human health and have been reported to have vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immunostimulating, antiallergic and antiviral effects (Middletone and Kandaswami, 1992; Yamamoto and Gaynor. 2001; Cushnie and Lamb, 2005; 2011), anti-cancer (deSousa *et al.*, 2007) and anti-diarrheal activities (Schuier *et al*., 2005). Flavonoids and their relative compounds are found to exhibit numerous biological activities like scavenging hydroxyl radicals (Lean *et al*., 1999) and DPPH radical (Apati *et al*., 2003). The present study is aimed to evaluate the flavonoid compound profile in the whole-plant methanol extracts 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 (Gamble, 1956), Indian Medicinal Plants (Kirtikar and Basu, 2003) 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 flavonoid compounds.

HPTLC analysis for flavonoids:

- ***Test solution***: Methanol extracts of *P. chinense, P. glabrum* and *P. barbatum*.
- ***Standard solution***: Methanol.
- ***Standard chemical***: RUT-Rutin (for *P. chinense*-X3/*P. glabra*-X-4) and APN-Apigenin (for *P. barbatum*-Y3) were used as reference standard compound.
- ***Mobile phase***: Ethyl acetate-Butanone-Formic acid-Water (5: 3: 1: 1).
- ***Spray reagent***: 1% ethanolic aluminium 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 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 (Shah *et al*., 2008).

Results and Discussion

The chromatogram (Fig. 1) shows flavonoid profile of whole-plant methanolic extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* – Y3) and is compared with flavonoid standards. Yellow coloured fluorescent zones present in the rutin and

apigenin standards and plant sample tracks scanned at UV 366nm and 254nm modes were observed in the chromatogram after derivatization and this may be confirmed the presence of flavonoid compounds (Fig. 1) in the *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3).

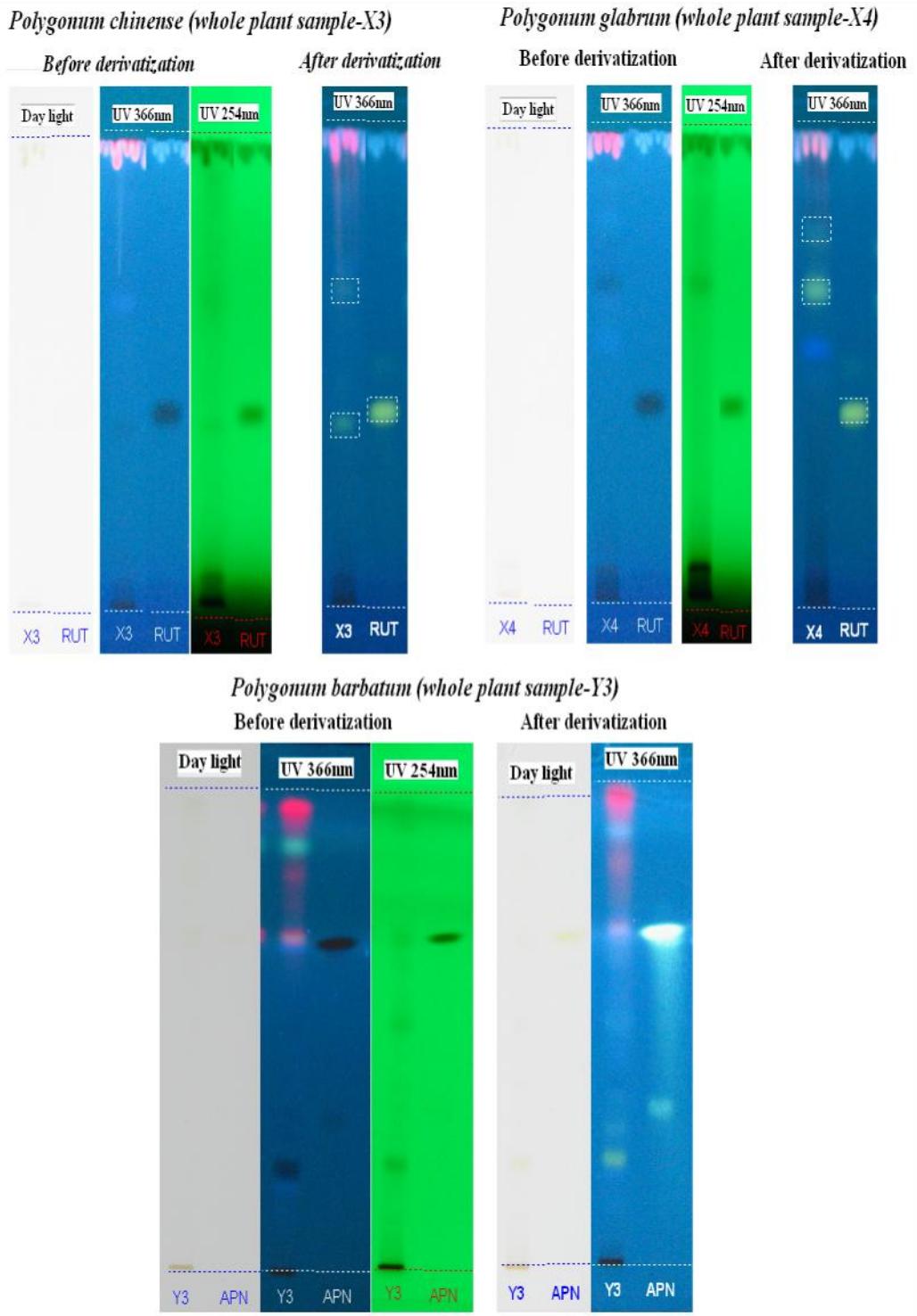

Figure 1: Chromatogram for flavonoid compounds in the whole plant methanol extract of *Polygonum* species.

HPTLC analysis for flavonoid profile in the whole-plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) shows several peaks (R_f -values) of compounds (Tab. 1; Fig. 2) and were compared with rutin and apigenin standards. The densitogram (Fig. 2) shows the profile of flavonoid compounds present in the whole plant methanolic extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and rutin standard for *P. chinense/P. glabrum* and apigenin standard for *P. barbatum* samples scanned at 366nm and 254nm, respectively.

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

***P. chinense* (X3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X3	1	0.01	96.2	466.3	Unknown
X3	2	0.07	25.1	294.1	Unknown
X3	**3**	**0.38**	**63.7**	**1770.5**	**Flavonoid 1**
X3	4	0.66	47.9	2441.2	Flavonoid 2
X3	5	0.71	15.7	259.2	Unknown
X3	6	0.91	19.9	506.1	Unknown
X3	7	0.95	52.1	709.0	Unknown
X3	8	0.98	40.6	452.7	Unknown
***P. glabrum* (X4)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X4	1	0.06	106.1	1633.9	Unknown
X4	2	0.67	143.6	6502.6	Flavonoid 1
X4	3	0.78	35.1	1593.4	Flavonoid 2
X4	4	0.83	41.6	1556.8	Unknown
X4	5	0.92	31.2	742.2	Unknown
X4	**6**	**0.97**	**114.4**	**2555.4**	**Unknown**
***P. barbatum* (Y3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
Y3	1	0.10	18.4	292.7	Unknown
Y3	2	0.22	183.1	5934.5	Flavonoid 1
Y3	3	0.34	21.2	546.3	Unknown
Y3	**4**	**0.38**	**27.9**	**725.2**	**Flavonoid 2**
Y3	5	0.51	107.1	4254.2	Unknown
Y3	6	0.64	45.3	1046.2	Unknown
Y3	7	0.70	131.9	6162.0	Unknown
Y3	8	0.77	79.0	3418.3	Unknown
Y3	9	0.84	24.4	825.5	Unknown
Y3	**10**	**0.97**	**154.1**	**6260.5**	**Unknown**
Control-1(X3/X4)	**1**	**0.40**	**336.4**	**11363.3**	**Rutin standard**
Control-2 (Y3)	**1**	**0.70**	**663.7**	**14855.4**	**Apigenin standard**

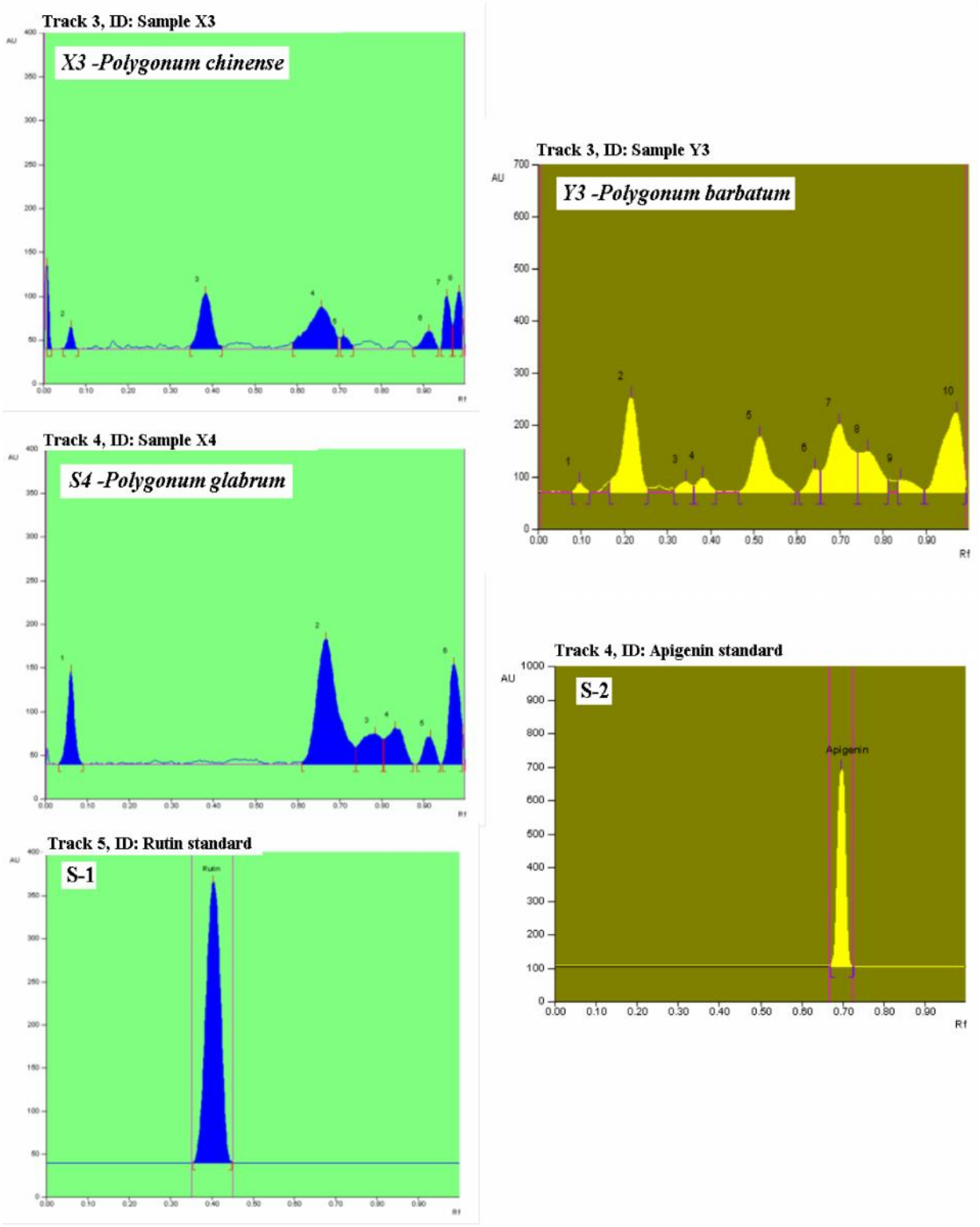

Figure 2: Densitogram showing the HPTLC analysis of flavonoid compounds in the whole plant methanolic extracts of *Polygonum* species (X3/X4/Y3); and Rutin standard 'S-1' (for X3/ X4) scanned at 366nm and Apigenin standard 'S-2' (for Y3) scanned at 254nm.

The 3D display of densitogram for flavonoid profile shows all tracks of *Polygonum* species (*P. chinense* – X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and rutin standard for *P. chinense/P. glabrum* and apigenin standard *P. barbatum* samples scanned at 366nm and 254nm, respectively (Fig. 3).

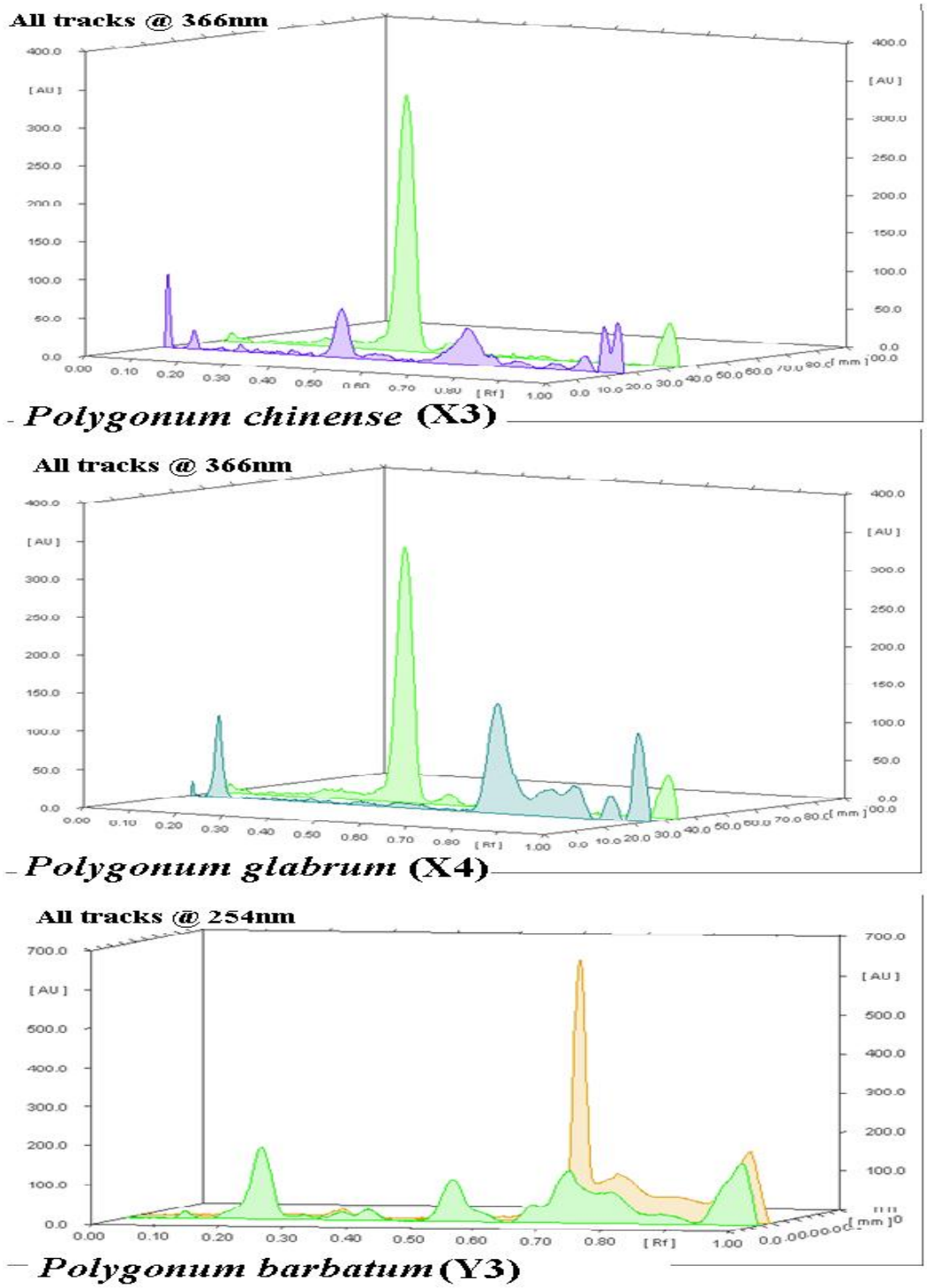

Figure 3: HPTLC densitogram 3D display of all tracks for flavonoid compounds in the whole plant methanolic extract of *Polygonum* species (X3/ X4/ Y3) and Standards (Rutin for X3/X4; and Apigenin for Y3).

The whole-plant methanol extract of *P. chinense* (X3) shows 8 compounds with peak R_f values ranging from 0.01 to 0.98, peak height ranging from 15.7 to 96.2 and peak area ranging from 259.2.8 to 2441.2 as compared to rutin standard (0.40, 336.4 and 11363.3, respectively). Among the 8 compounds detected, 2 were identified as flavonoids (peak no. 3 & 4) and the others were unknown (Tab. 1-X3; Fig. 2-X3).

Similarly, the methanol extract of *P. glabrum* (X4) shows 6 compounds with varied peak R_f values (0.06-0.97), peak height (31.2-143.6) and peak area (742.2-6502.6) as compared to rutin standard (0.40, 336.4 and 11363.3, respectively). Out of six compound detected, two compounds (peak No. 2 & 3) were identified as flavonoids and the others were unknown ((Tab. 1-X4; Fig. 2-X4)).

On the other hand, the whole-plant methanol extract of *P. barbatum* (Y3) shows 10 compounds (Tab. 1-Y3) with peak R_f values ranging from (0.10 & 0.97, peak height from 18.4 to 183.1 and peak area from 292.7 to 6260.4 as compared to rutin standard (0.70, 663.7 and 14855.4, respectively) and out of 10 compounds detected, one compound (peak No. 2) is identified as flavonoid and all others were unknown (Tab. 1-Y3; Fig. 2-Y3).

In general, one flavonoid compound (peak No. 3 of X3 and peak No 4 of Y3) detected in the *P. chinense* and *P. barbatum* shows similar peak R_f value (0.38). Similarly, *P. glabrum* and *P. barbatum* show one identical unknown compound (peak No. 6 of X4 and peak No. 10 of Y3) with similar peak R_f value (0.97) (Tab. 1; Fig. 2), while all other compounds of *Polygonum* species shows no similarities in their peak R_f values of compounds detected.

Among the chemical constituents recognized in the *Polygonum* species, flavonoids are the most common constituents found in *Polygonum* and have previously been used as chemotaxonomic markers of the genus (Bidywt *et al*., 2002). Flavonoids exists in numerous plants were detected in the roots and leaves of *Polygonum cuspidatum* (Sun and Sneeden, 1999; State Administration of Traditional Chinese Medicine, 1999; Xiao *et al*., 2002). The efficiency of phenol substances including flavonoids act as antioxidant has been reported (Liu *et al*., 2003; Cai *et al*., 2006). In this study, the methanol extracts of *Polygonum species* make certain the presence of flavonoids and the number and nature of flavonoid compounds of three *Polygonum species* tested differ from one another.

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