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Research Article

HPTLC Evaluation of Alkaloids Profile in the Methanol Extracts of three *Polygonum* species

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Abstract

HPTLC analysis was carried out to assess alkaloid 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 10, 12 and 9 compounds, respectively, and were compared with colchicine standard. Among the compounds, 1, 3 and 1 compounds in each sample were identified as alkaloids while the others were unknown. One unknown compound each from *P. chinense* and *P. glabrum* showing same peak R_f values (0.65). Similarly, two alkaloid compounds from *P. glabrum* and *P. barbatum* also showed same peak R_f values (0.28 & 0.81), while all other detected compounds of *Polygonum* species shows no similarities in their peak R_f values. The results of present study indicate that the nature and number of alkaloid compounds differ among the *Polygonum* species tested.

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

Introduction

Alkaloids are a group of nitrogen-containing bases, most of which are drugs. Only a few (like caffeine) are derived from purines or pyrimidines, while majority is produced from amino acids. Alkaloid-containing plants have been used by human beings since ancient times for therapeutic and recreational purposes. More than 15,000 naturally occurring alkaloids (mostly of herbal origin) have been found so far, and the number is increasing faster and faster. Although alkaloids often display unspecific biological activity, just this has rarely turned out to be of therapeutic value. Even though only few genuine alkaloids are in use, many alkaloid derivatives are important as drug leads (Newman *et al*., 2007). Many alkaloids are still used in medicine, usually in the form of salts. The present study is aimed to understand the alkaloid compound profile in the whole-plant samples of three *Polygonum* species.

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 alkaloid compounds.

HPTLC analysis for Alkaloids

- ***Test solution***: Methanol extracts of *P. chinense, P. glabrum* and *P. barbatum*.
- ***Standard solution***: Methanol.
- ***Standard chemical***: Colchicine (COL) was used as reference standard compound.
- ***Mobile phase***: Ethyl acetate-Methanol-Water (100: 13.5: 10).
- ***Spray reagent***: Dragendorff's reagent followed by 10% sodium nitrite 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 alkaloid profile of whole-plant methanol extracts of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and is compared with colchicine standard. Black coloured quenching zone at UV 254nm was present in the colchicine standard (before and after derivatization) and bright orange and brown colored zones at day light mode were present in the *Polygonum* species sample tracks observed in the chromatogram after derivatization, confirmed the presence of alkaloid in the colchicine standard and *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) samples (Fig. 1). HPTLC analysis for alkaloid 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 compared with colchicine standard.

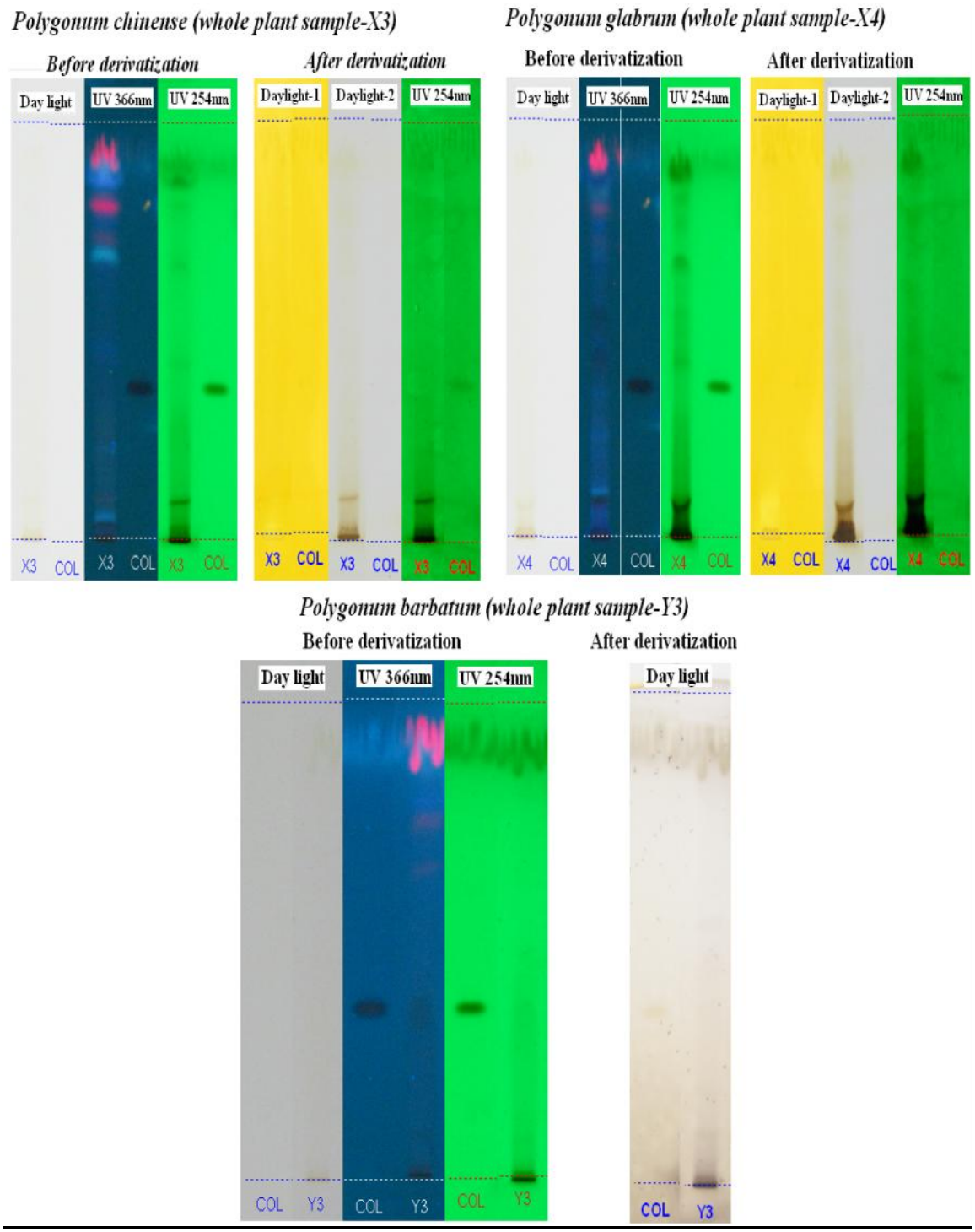

Figure 1: Chromatogram for alkaloid compound in the whole plant methanol extract of *Polygonum* species.

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

***P. chinense* (X3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X3	1	0.03	37.3	510.9	Unknown
X3	2	0.09	251.8	3648.0	Alkaloid 1
X3	3	0.23	42.3	885.1	Unknown
X3	4	0.27	40.9	1949.5	Unknown
X3	5	0.42	54.3	1512.3	Unknown
X3	**6**	**0.65**	**111.4**	**6119.5**	**Unknown**
X3	7	0.76	70.8	2031.3	Unknown
X3	8	0.79	107.6	3420.3	Unknown
X3	9	0.85	343.3	10843.3	Unknown
X3	10	0.89	265.4	12112.8	Unknown
***P. glabrum* (X4)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X4	1	0.07	158.3	4525.8	Alkaloid 1
X4	2	0.15	10.2	98.7	Unknown
X4	3	0.21	19.5	486.4	Unknown
X4	**4**	**0.28**	**25.8**	**835.7**	**Alkaloid 2**
X4	5	0.41	89.8	3631.9	Unknown
X4	6	0.48	67.8	2413.4	Unknown
X4	7	0.53	76.4	2580.4	Unknown
X4	8	0.58	54.0	1599.1	Unknown
X4	**9**	**0.65**	**193.3**	**7037.7**	**Unknown**
X4	10	0.74	129.9	6472.0	Alkaloid 3
X4	**11**	**0.81**	**187.3**	**6530.0**	**Unknown**
X4	12	0.87	451.6	26952.4	Unknown
***P. barbatum* (Y3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
Y3	1	0.08	24.2	434.9	Unknown
Y3	**2**	**0.28**	**32.9**	**1063.3**	**Alkaloid 1**
Y3	3	0.34	53.5	1864.4	Unknown
Y3	4	0.35	53.2	1481.3	Unknown
Y3	5	0.40	24.0	523.9	Unknown
Y3	6	0.47	29.2	1061.3	Unknown
Y3	7	0.75	10.1	206.3	Unknown
Y3	**8**	**0.81**	**62.8**	**1690.5**	**Unknown**
Y3	9	0.91	307.1	23332.7	Unknown
Control-1 (X3/X4)	**1**	**0.35**	**486.1**	**11466.9**	**Colchicine standard**
Control-2 (Y3)	**1**	**0.36**	**555.0**	**12312.3**	**Colchicine standard**

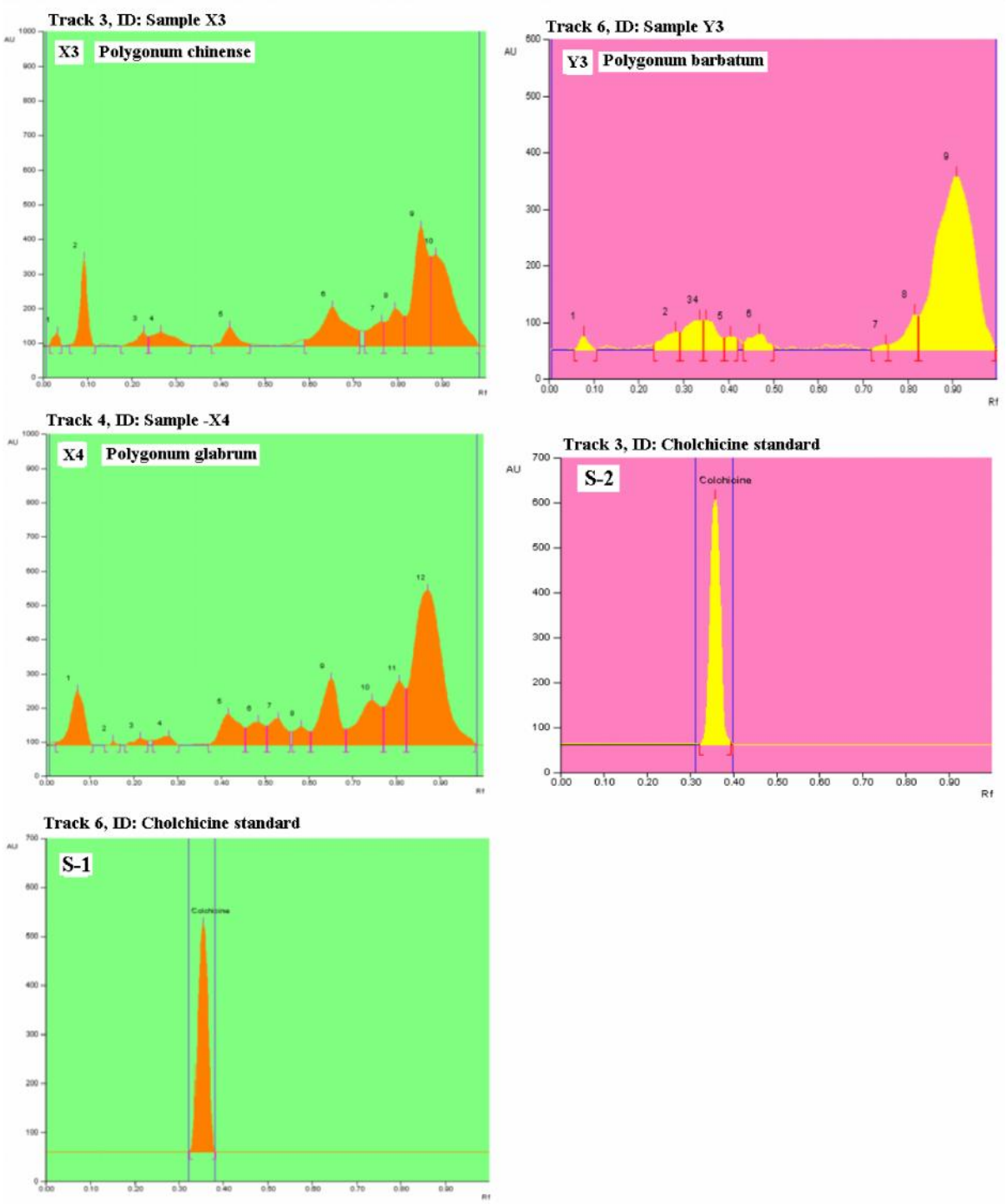

Figure 2: Densitogram showing the HPTLC analysis of alkaloid compounds in the whole plant methanolic extracts of *Polygonum* species (X3/X4/Y3); and Colchicine standards 'S-1' (for X3/X4) and 'S-2' (for –Y3) scanned at 254nm.

The densitogram (Fig. 2) shows the profile of alkaloid compounds present in the whole-plant methanol extract of *P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3 samples scanned at 254nm. The 3D display of densitogram for alkaloid profile shows all tracks of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) samples and colchicine standard scanned at 254nm (Fig. 3).

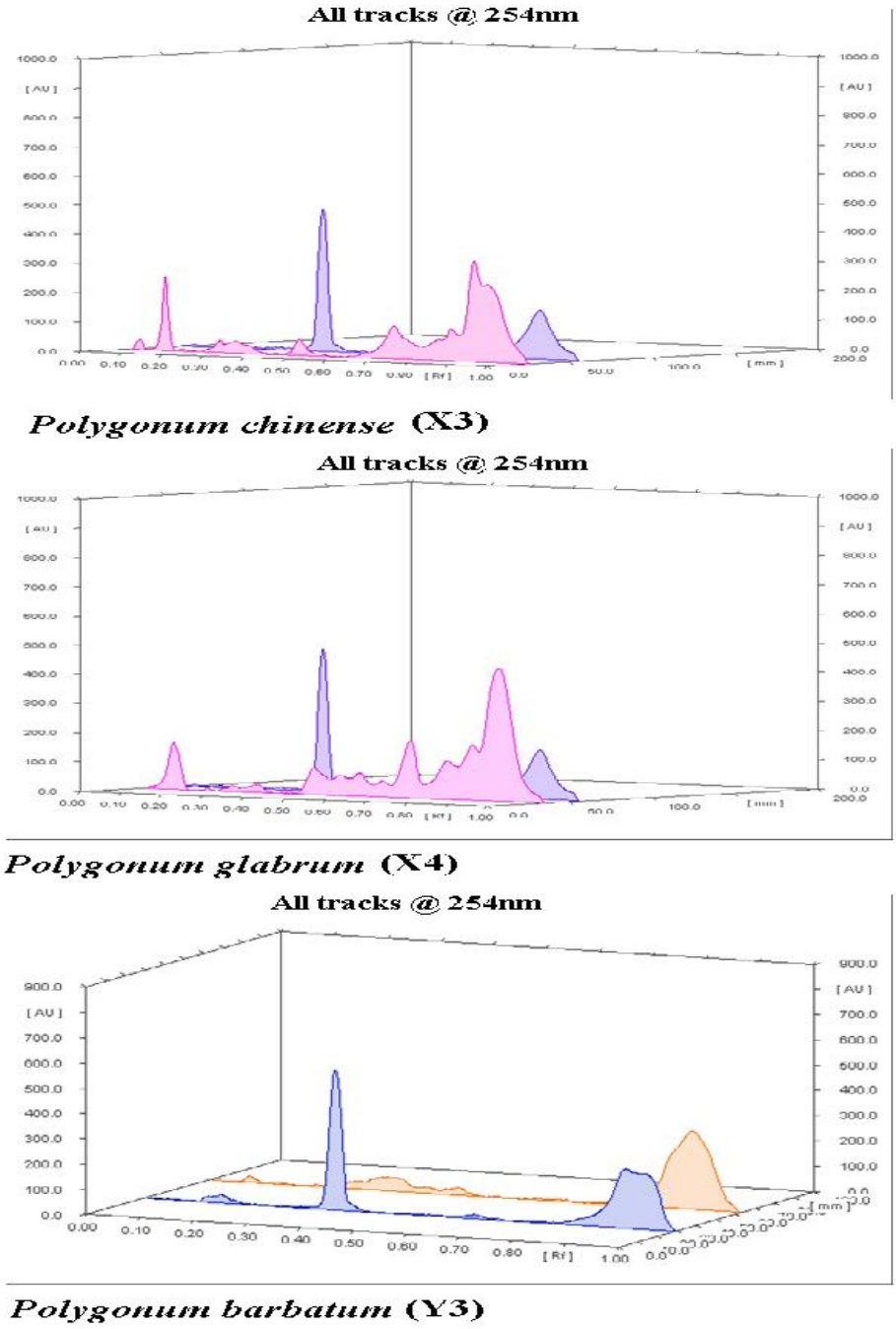

Figure 3: HPTLC densitogram 3D display of all tracks for alkaloid compounds in the whole plant methanolic extract of *Polygonum* species (X3/ X4/ Y3) and standard (Colchicine).

The whole-plant methanol extract of *P. chinense* (X3) shows ten compounds (Tab. 1-X3) with peak R_f values ranging from 0.03 to 0.89, peak height ranging from 37.3 to 343.3 and peak area ranging from 510.9 to 12112.8 as compared to standard (0.35, 486.1 and 11466.9, respectively). Among the 10 compounds, one was identified as alkaloid (peak no.2) and the others were unknown (Tab. 1-X3; Fig. 2-X3).

Polygonum glabrum (X4) whole-plant methanol extract shows 12 compounds (Tab. 1-X4) with different peak R_f values (0.07-0.87), peak height (10.2-451.6) and peak area (98.7-26952.4) as compared to colchicine standard (0.35, 486.1 and 11466.9, respectively). Out of 12 compounds detected, two (No. 1 & 10) was identified as alkaloid and the others were unknown (Tab. 1-X4; Fig.2-X4).

The whole-plant methanol extract of *P. barbatum* (Y3) contained nine compounds (Tab. 1-Y3) with peak R_f values ranging from 0.08 to 0.91, peak height from 10.1 to 307.1 and peak area from 206.3 to 23332.7 as compared to colchicine standard (0.36, 555.0 and 12312.3, respectively). Among the nine compound detected, one (peak No. 2) was identified as alkaloid and all the other compounds were unknown (Tab. 1-Y3; Fig. 2-Y3).

Among the whole-plant methanol extracts of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) tested, one unknown compound detected from *P. chinense* (peak No. 6) and *P. glabrum* (peak No. 4) shows similar R_f values (0.28), while two unknown compounds detected from *P. glabrum* (peak No. 4 & 11) and *P. barbatum* (peak No. 2 & 8) shows similar R_f values (0.28 & 0.81, respectively). There is no similarity in their compounds detected between *P. chinense* and *P. barbatum*.

The alkaloid extracts of plants had showed a strong antioxidant activity, especially a strong radical scavenger power (Maiza-Benabdesselam *et al.*, 2007), so they could be used as good sources of natural antioxidants for medicinal and commercial needs. In this study, the HPTLC analysis of methanol extracts of *Polygonum* species whole-plant samples make certain the presence of alkaloids and the nature and number of alkaloids present in these three *Polygonum* species may differ from one another.

Acknowledgments

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