COMPARATIVE ANALYSIS OF TERPENOID COMPOUND PROFILE BY HPTLC IN THREE POLYGONUM SPECIES

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Abstract

HPTLC analysis was carried out on terpenoid 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 8, 10 and 9 compounds, respectively, and were compared with solanesol standard. Among the compounds, 7 compounds in each sample were identified as terpenoids while the others were unknown. Two terpenoid compounds each from P. chinense and P. glabrum showing same peak Rf values (0.41 & 0.77). Similarly, another one unknown compound of P. chinense and P. barbatum also showed same peak Rf values (0.11), while all other detected compounds of Polygonum species showed no similarities in their peak Rf values. The HPTLC analysis of methanol extracts of Polygonum species shows variations in the nature and number of terpenoid compounds.

Key words: Polygonum species, Whole-plant samples, Methanol extracts, HPTLC analysis, Terpenoid compounds.

I. INTRODUCTION

Terpenoids also called “isoprenoids” constitute one of the largest families of natural products. It accounts for more than 40,000 individual compounds of both primary and secondary metabolisms. Most of them are of plant origin, and hundreds of new structures are reported every year¹-³. All organisms naturally produce some terpenoids as part of primary metabolism, but many produce terpenoids via secondary metabolism. Plant terpenoids are used extensively for their aromatic qualities and play important role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical functions. Polygonum is a genus in the Polygonaceae family having many medicinal properties. In Chinese medicine, Polygonum extracts used to treat urinary infection⁴. 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 therapy⁵ and in the perfume industry⁶. Polygonum species has also been reported to possess several pharmacological properties like antimicrobial activity⁷, cytotoxic activity against HeLa (human cervical carcinoma)⁸, antioxidant activity⁹ and anticancer activity¹⁰,¹¹. In the present study, it is aimed to evaluate the terpenoid compounds profile in the whole-plant samples of three Polygonum species – P. chinense, P. glabrum and P. barbatum.

II. 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\(^\text{12}\), Indian Medicinal Plants\(^\text{13}\) 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 used 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 terpenoid compounds.

2.5.1. HPTLC analysis for terpenoids

- **Test solution**: Methanol extracts of *P. chinense*, *P. glabrum* and *P. barbatum*.
- **Standard solution**: Methanol.
- **Standard chemical**: SOL–Solanesol was used as reference standard compound.
- **Mobile phase**: n-Hexane-Ethyl acetate (7.2: 2.9).
- **Spray reagent**: Anisaldehyde sulphuric acid reagent.

2.5.2. 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.

2.5.3. 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 90 mm.

2.5.4. 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.

2.5.5. 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 photodocumentation (CAMAG REPROSTAR 3) chamber.

2.5.6. 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\(^{14}\).
III. RESULTS AND DISCUSSION

The chromatogram (Figure 1) shows terpenoid profile of whole plant methanol extract of Polygonum species (P. chinense – X3, P. glabrum – X4 and P. barbatum – Y3) and is compared with solanesol standard. Blue-violet coloured zones at day light mode present in the solanesol standard and plant samples track were observed in the chromatogram after derivatization and this confirmed the presence of terpenoid compounds in the whole plant extracts of Polygonum species (P. chinense – X3, P. glabrum – X4 and P. barbatum – Y3) (Figure 1).

![Figure 1: Chromatogram for terpenoid compounds in the whole plant methanol extract of Polygonum species.](image)

The 3D display of densitogram for terpenoid profile shows all tracks of Polygonum species (P. chinense – X3, P. glabrum – X4 and P. barbatum – Y3) and solanesol standard scanned at 500nm (Figure 2).

![Figure 2: HPTLC densitogram 3D display of all tracks for terpenoid compounds in the whole plant methanol extract of Polygonum species (X3/X4/Y3) and Standards (Solanesol for X3/X4/Y3).](image)

The densitogram (Figure 3) showed the profile of terpenoid compounds present in the whole plant methanol extract of Polygonum species (P. chinense – X3, P. glabrum – X4 and P. barbatum – Y3); and solanesol standard scanned at UV 500nm.
Figure 3: Densitogram showing the HPTLC analysis of terpenoid compounds in the whole plant methanol extracts of Polygonum species (X3/X4/Y3); and Solanesol standard ‘S-1’ (for X3/X4) scanned at 500nm and Solanesol standard ‘S-2’ (for Y3) scanned at 500nm.

HPTLC analysis for terpenoid profile in the whole plant methanol extract of Polygonum species (P. chinense –X3, P. glabrum –X4 and P. barbatum –Y3) showed several peaks (Rf-values) of compounds (Table 1; Figure 3) and were compared with solanesol standard.

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

<table>
<thead>
<tr>
<th>P. chinense (X3)</th>
<th>Peak</th>
<th>Rf</th>
<th>Height</th>
<th>Area</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>X3</td>
<td>1</td>
<td>0.11</td>
<td>12.8</td>
<td>320.5</td>
<td>Terpenoid 1</td>
</tr>
<tr>
<td>X3</td>
<td>2</td>
<td>0.31</td>
<td>103.3</td>
<td>3562.5</td>
<td>Terpenoid 2</td>
</tr>
<tr>
<td>X3</td>
<td>3</td>
<td>0.36</td>
<td>117.4</td>
<td>3348.5</td>
<td>Terpenoid 3</td>
</tr>
<tr>
<td>X3</td>
<td>4</td>
<td>0.41</td>
<td>77.3</td>
<td>2929.1</td>
<td>Terpenoid 4</td>
</tr>
<tr>
<td>X3</td>
<td>5</td>
<td>0.47</td>
<td>43.1</td>
<td>1140.8</td>
<td>Terpenoid 5</td>
</tr>
<tr>
<td>X3</td>
<td>6</td>
<td>0.59</td>
<td>31.9</td>
<td>1662.7</td>
<td>Unknown</td>
</tr>
<tr>
<td>X3</td>
<td>7</td>
<td>0.73</td>
<td>47.8</td>
<td>192.8</td>
<td>Terpenoid 6</td>
</tr>
<tr>
<td>X3</td>
<td>8</td>
<td>0.77</td>
<td>59.4</td>
<td>2273.5</td>
<td>Terpenoid 7</td>
</tr>
</tbody>
</table>

P. glabrum (X4) | Peak | Rf  | Height | Area  | Assigned substance |
<table>
<thead>
<tr>
<th></th>
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<tr>
<td>X4</td>
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<tr>
<td>X4</td>
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<tr>
<td>X4</td>
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<tr>
<td>X4</td>
<td>7</td>
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The methanol extract of *P. chinense* (X3) showed 8 compounds with peak Rf values ranging from 0.11 to 0.77, peak height ranging from 12.8 to 117.4 and peak area ranging from 192.8 to 3565.5 as compared to solanesol standard (0.46, 434.3 and 14285.0, respectively). Out of 8 compounds detected, 6 compounds (peak No. 2-4, 7 & 8) were identified as terpenoids and others were unknown (Table 1-X3; Figure 3-X3).

The whole plant methanol extract of *P. glabrum* (X4) showed 10 compounds with varied peak Rf values (0.03-0.77), peak height (13.7-90.5) and peak area (118.3-2291.1) as compared to solanesol standard (0.48, 434.3 and 14285.0, respectively). Out of 10 compounds detected, 7 compounds (peak no. 2-6, 9 & 10) were identified as terpenoids and others were unknown (Table 1-X4; Figure 3-X4).

*Polygonum barbatum* (Y3) whole plant methanol extract showed 9 compounds (Table 1-Y3) with peak Rf values ranging from (0.11 to 0.92, peak height from 27.1 to 147.9.5 and peak area from 614.0 to 5365.2 as compared to solanesol standard (0.74, 400.9 and 10819.7, respectively) and out of 9 compounds, 7 compounds (peak No. 1, 4-9) were identified as terpenoids and the remaining were unknown (Table 1-Y3; Figure 3-Y3).

In general, the two terpenoid compounds (peak No. 4 & 8) of *P. chinense* and of *P. glabrum* (peak No. 6 & 10) showed same peak Rf values (0.41 & 0.77, respectively). Another one terpenoid compound (peak No. 1) of *P. chinense* and of *P. barbatum* (peak No. 1) was also showed similar peak Rf values (0.11). On the other hand, none of the compounds detected in the *P. glabrum* and *P. barbatum* showed similar peak Rf values (Table 1; Figure 3).

The results of present study indicate that the HPTLC analysis of methanol extracts of Polygonum species make certain the presence of terpenoid compounds and the nature and number of terpenoids present in the polygonum species is varied.

**IV. ACKNOWLEDGMENT**

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**BIBLIOGRAPHY**


