HPTLC Fingerprinting of different leaf extracts of Tylophora indica (Burm f.) Merill.

Gupta Mayank¹*, Singh Mhaveer², Mukhatr Hayat M.¹, Ahmad Sayeed²
¹SBS College of pharmacy, Patti, Punjab-143416, India.
²Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India.
* Corresponding Author Mayank Gupta 263, Vidya Vihar, West Enclave, Pitampura,
New Delhi-110034. Email- Mayank.pharma@yahoo.com, Mayank_pharmaceuticals@yahoo.co.in.

ABSTRACT

Tylophora indica is very popularly used for the treatment of asthma based on its traditional use for asthma. Tylophora is a perennial climbing plant native to the plains, forests, and hills of southern and eastern India. A method has been developed for different extracts of Tylophora indica for HPTLC fingerprinting analysis for identification and quantification of marker compound. For chloroform extract-Chloroform(90): Methanol (5) : Ethyl acetate (5) v/v, Methanol Extract-Toluene(5): Chloroform(90), Ethyl acetate(5) v/v and for Petroleum ether extract-Hexane(40) : Ethyl acetate (60) v/v. The HPTLC fingerprinting profile developed for different extracts of Tylophora indica will help in proper identification and quantification of marker compound.

INTRODUCTION

Tylophora indica (Burm f.) Merill. (Family: Asclepidaceae) commonly known as Antmul is a twining perennial plant distributed throughout southern and eastern part of India in plains, forests, and hilly places. The plant is found growing normally in Uttar Pradesh, Bengal, Assam, Orissa, Himalayas and sub Himalayas in India. It is a branching climber or shrub that grows up to 1.5 meters, leaves are obvate-oblong to elliptic-oblong, 3-10cm long and 1.5-7cm wide. Roots Long fleshy with longitudinally fissured light brown, corky bark. Flowers minute, 1-1.5 cm across, in 2-3 flowered fascicles in axillary umbellate cymes. Calyx divided nearly to the base, densely hairy outside; segments lanceolate, acute. Corolla greenish yellow or greenish purple; lobes oblong, acute. Fruit a follicle, up to 7 x 1cm, ovoid lanceolate, tapering at apex forming fine micro, finally striate, glabrous, Seeds 0.6-0.8 x 0.3-0.4cm long.

The plant has been reported to contain 0.2-0.46% alkaloids viz. Tylophorine, tylophorinine, tylophorinidine, (+)septicine, isotypocrebrine, tylophorinicine, sterols, flavanoids, wax, resins, and tannins. The plant has been traditionally used for the treatment of bronchial asthma, jaundice and inflammation. Its antitumor, immunomodulatory, antioxidant, antiasthmatic, smooth muscle relaxant, antihistaminic, hypotensive, antihyperemic activities are scientifically proven. In Ayurveda, the plant has been used in treatment of asthma, dermatitis and rheumatism. Although the leaf and root of this plant are widely used for treating jaundice in Northern Karnataka, there is a paucity of scientific evidence regarding its usage in liver disorder. The other reported activities include immunomodulatory activity, anti-inflammatory activity, anticancer activity and antiamoebic activity.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of Tylophora indica is collected in the month of August-September (2009) from Herbal Garden of JAMIA HAMDARD, New Delhi was identified and authenticated at Raw material, Herbarium and museum NISCAIR, CSIR, New Delhi, India and sample was submitted in museum for future reference. Ref. NISCAIR/RHMD/consult/-2009-10/1361/163.
Preparation of extract

For development of HPTLC fingerprints the different extracts of *Tylophora indica* were prepared by taking 5 gm of dried leaves powder in 250 ml of conical flask and added 100 ml of corresponding solvents such as methanol Chloroform and Pet. ether and heated on water bath for one hour. Filtered the extract and evaporated to dryness and prepared the samples 20 mg/ml by reconstitute with same solvents and developed solvent systems for their separation by thin layer chromatography.

TLC/HPTLC Profile

Developing solvent system

A number of solvent systems were tried, for extracts Methanolic extract, Chloroform extract, and Petroleum ether extract. For chloroform extract-Chloroform(90): Methanol (5) : Ethyl acetate (5) v/v, Methanol Extract-Toluene(5): Chloroform(90), Ethyl acetate(5) v/v and for Petroleum ether extract-Hexane(40) : Ethyl acetate (60) v/v. The solvent system tried for Pet. Ether extract 12 peaks is observed, in methanolic extract 12 peaks is obtained too. However in Chloroform 8 peaks is observed. The RF values obtained are calculated through WINCATS HPTLC software supplied with the instrument (Table 1, 2 &3).

Sample application

Application of bands of each extract was carried out (5mm in length and 2 μl in concentration) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F 
254 aluminium sheets (20x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WINCATS software.

Development of chromatogram

After the application of spots, the chromatogram was developed in Twin trough glass chamber 20x 10 cm saturated with solvents developed for each extracts before putting the plates for 20 mins.

Detection of spots

The air-dried plates were viewed in ultraviolet radiation to mid day light. The chromatograms were scanned by densitometer at 366 nm. The Rf values and fingerprint data were recorded by WIN CATS software. The data obtained are summarized in Table (1, 2 &3) for where as the developed chromatogram can be seen at 366 nm.

RESULTS AND DISCUSSION

Different solvent systems were tried by hit and trial method, for extracts: Methanolic extract, Chloroform extract, and Petroleum ether extract. Satisfactory resolution was obtained in solvent systems developed, for chloroform extract-Chloroform(90): Methanol (5) : Ethyl acetate (5) v/v, Methanol Extract-Toluene(5): Chloroform(90), Ethyl acetate(5) v/v and for Petroleum ether extract-Hexane(40) : Ethyl acetate (60) v/v. The solvent system tried for Pet. Ether extract 12 peaks is observed, in methanolic extract 12 peaks is obtained too. However in Chloroform 8 peaks is observed. The RF values obtained are calculated through WINCATS HPTLC software supplied with the instrument (Table 1, 2 &3).
HPTLC Fingerprinting of different leaf extracts of Tylophora indica (Burm f.) Merill.

Table 1 Thin layer chromatography of Pet. Ether ext.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent System developed</th>
<th>No. of peaks and Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet. Ether</td>
<td>Hexane(40):ethyl acetate(60)</td>
<td>(12) 0.01, 0.07, 0.13, 0.22, 0.27, 0.29, 0.37, 0.42, 0.53, 0.61, 0.85, 0.94</td>
</tr>
</tbody>
</table>

For Methanolic extract:

Figure 3 Pet. Ether Ext. peaks at 366nm.

Figure 4 Methanolic Ext. 3-D View at 366nm.

Figure 5 Developed TLC plate of methanolic extract of Tylophora indica at 366nm
**Table 2 Thin Layer chromatography of methanolic ext.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent system developed</th>
<th>No. of peaks and Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic Extract</td>
<td>Toluene(5):Chloroform(90):ethylacetate(5)</td>
<td>(12) 0.01, 0.04, 0.11, 0.17, 0.24, 0.31, 0.37, 0.45, 0.53, 0.60, 0.83, 0.92</td>
</tr>
</tbody>
</table>

**Figure 6** Methanolic Ext. peaks at 366nm.

**Figure 7** Chloroform extract 3-D view at 366nm.

**Figure 8** Developed TLC plate of chloroform extract of *Tylophora indica* at 366nm.
HPTLC Fingerprinting of different leaf extracts of Tylophora indica (Burm f.) Merill.

Table 3 Thin Layer chromatography of Chloroform ext.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent system developed</th>
<th>No. of peaks and Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform Ext</td>
<td>Chloroform (90): methanol (5): ethyl acetate (5)</td>
<td>(8) 0.03, 0.52, 0.59, 0.66, 0.74, 0.78, 0.86, 0.95</td>
</tr>
</tbody>
</table>

CONCLUSION

Medicinal Plant material is obtained from different heterogeneous sources which may lead to variation in therapeutic values and variation in phytochemistry. The HPTLC-Fingerprinting profile is a very important parameter of herbal drug standardization for the proper identification for medicinal plants. This parameter can also be very important tool if adulteration is suspected in medicinal plant material. The present HPTLC-fingerprinting profile can be used as a diagnostic tool for the identity and to determine the quality and purity of the plant material in future studies. Also the present study on Tylophora indica leaves will help in identification and quantification of chemical marker compound.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Sayeed Ahmad, Assistant professor, Department of Pharmacognosy, Faculty of Pharmacy, Jamia Hamdard, New Delhi, for providing laboratory facilities and reagents and chemicals.

REFERENCES

6.) Chopra IC, Chopra RN, Nayar SL, Glossary of Indian medicinal plants, CSIR, New Delhi, 1986.