“HPTLC” STUDY OF YASTIMADHU (Glycyrrhiza glabra)-
AN APPROACH FOR RAW DRUG STANDARDIZATION

Indrajit Singh Kushwaha¹, B. Behera ², V. Bhushan ³, S. Mohapatra⁴
¹,² Assistant professor, Department of Dravyaguna,
³Professor & H.O.D., Department of Dravyaguna
⁴Assistant professor, Department of Rasa Shastra,
A&U Tibbia College, Government of NCT Delhi-5

ABSTRACT

Yastimadhu is an herbal material used in different compound formulations as well as single for treating various disease conditions. In the current study, the used parts of yastimadhu i.e. root is assessed for its HPTLC (High performance thin layer chromatography) findings. In the HPTLC study, the sample material is evaluated for chemical constituents present in yastimadhu (Glycyrrhiza glabra Linn) root. In the current study the chemical constituents found out are glycyrrhizin and glycyrrhizic. Yashtimadhu is rich source of glycyrrhizin and glycyrrhizic acid present in root which are responsible for its pharmacology. These chemicals could be the finger print for the particular test sample. The data obtained are discussed to lay out the possible way of raw drug standardization for herbal material. Hope this scientific write up will be a step ahead for drug standardization in Ayurvedic system of Medicine.

Key words-Yastimadhu, phytochemical, glycyrrhizin, mahakashaya
INTRODUCTION:

Plants synthesize a variety of phytochemicals that are useful for the maintenance of health in humans and other animals. Due to low toxicity and known pharmacological activity, Ayurvedic drugs have been popularly and extensively used for many centuries.

Yastimadhu is a shrub attaining a height up to 2m, leaves are multifoliate, Flower in axillary spikes and pharmacologically it possess madhura- rasa, guru and snigdha guna, sheeta veerya and madhura vipaka respectively as per various classical Ayurvedic texts[1-4]. It is used as an ingredient in many Ayurvedic medicines such as yastimadhu ghanavati, dhatri avaleha etc. In Charaka samhita, at the description of mahakshaya yastimadhu is given more emphasize and discussed for 11 times [5]. Now a day modern scientists are kin interest about the said plant due to its natural steroid contents and effective use in different autoimmune disorders [6].

Due to its applicability in many Ayurvedic formulations, adulterations of this highly potent material become very usual. This unlawful commercialization; in turn causes decreasing of the quality of the medicine. It is essential to standard the raw material for preparation of noble medicines.

For the authentication of the raw material, now a day’s different physico-chemical parameters are used for its quality assessment. To establish the fingerprint of a particular herbal material, its phyto-chemical findings of HPTLC is the basic tool. This tool is also facilitating the raw drug (herbal) standardization a step ahead. Though the identifying the study material i.e. yastimadhu has been defined in the ancient texts but for facilitating the cross disciplinary debate and for global acceptance, honest efforts have been made to assess it on the above said parameters and for establishing the data obtained.
MATERIALS AND METHOD:

The sample material i.e. *yastimadhu* is assessed for its phyto-chemical values specially HPTLC to establish the possible fingerprints for its authentication.

**Materials:**

Following materials are required for HPTLC analysis.

**Drug:**

The root powder of *yashtimadhu* is used for HPTLC. Alcoholic extract of *yashtimadhu* is used for this processure. The phytochemical analysis of the drug *yastimadhu* is carried out from the “Institute of pharmaceutical science” Jalandhar, in the supervision of director of this institute Dr. Anil Sharma. The selected drugs *yastimadhu* is subjected to the HPTLC analysis, Sample material i.e. *yastimadhu* is collected from authenticated shop in Delhi market. The root powder of *yashtimadhu* is used for HPTLC. Alcoholic extract of *yashtimadhu* is prepared in the laboratory.

**Chemicals and Reagents:**

- n-Butanol
- Glacial acetic acid
- Ethylene acetate
- Chloroform
- Methanol

**Apparatus required:**

- HPTLC Machine
- Handmade and cellulose plates
- Binding agent (starch).
- Cellulose ( microcrystalline)

- Cellulose (microcrystalline) with florescent indicator.
- Acetylated cellulose + CaSO4. ½ H2O
- Silica Gel.
- Glass support.
- Polyester (Plastic) sheets. (0.2 mm thick).
- Aluminium sheet (0.1 mm thick)
- Pre-coated of HPTLC Al sheets
  Silica gel 60 F254, Camag Cat No. 034.5554

**Method:-**

For doing HPTLC study of *Yastimadhu* different steps are followed having specific significance.

- Selection of HPTLC plates and solvents
- Sample preparation including any clean up
- Derivatisation
- Application of sample
- Development of chromatographic layers
- Detection including post chromatographic derivatisation
- Analysis and documentation of findings

A standard HPTLC machine having required features is selected for the study. After this, for preparing study sample 03gm. of *yashtimadhu* churna was dissolved in 20 ml of methanol. It was
stirred intermittently for 6 hours. The solution thus prepared was kept for 18 hours in standstill. Then it was filtered and filtered extract (filtrate) was used as original sample for HPTLC analysis.

**Stationary phase:**

TLC Al sheets Silica gel 60 F254 pre-coated Camag Cat No. 034.5554, cut to 10cm x 10cm

**Sample application – CAMAG Linomat 5**

**Instrument** CAMAG Linomat 5 “Linomat 5 080222” S/N 080222 (1.00.12) Executed by CT Institute of Pharmaceutical Science, Jalandhar.

**Linomat 5 application parameters**

Spray gas: Inert gas
Sample solvent type: Methanol
Dosage speed: 150nl/s
Predosage volume: 0.2 ul

**Sequence**

Syringe size: 100 µl
Number of tracks: 12
Application position: 8.0 mm
Band length: 8.0 mm

**Mobile phase:**

For Yashtimadhu- n-Butanol: Water : Glacial acetic acid - 7 : 2 : 1

1. **Development chamber:**
   Camag Twin Trough chamber of 10 x 10 cm with 3.5 s.s lid.

2. **Chamber Saturation:**
   20 minutes with paper

3. **Plate Equilibrium:** None

4. **Sample/Standard application:**
   Apply with the help of Camag ATS-4 of sample solution on pre-coated layer 10mm from the bottom edge.
   Band length 8mm.

5. **Development distance:**
   80mm

6. **Visualization:**
   Observe under UV cabinet at 254 nm

7. **Photo documentation**
   At 254 nm for Yastimadhu Visible

8. **Measurement Mode:**
   UV absorbance / reflectance

9. **Scanning:**
   a) **For Qualification:**
      Using Camag Scanner 3 with Win CATS software, Slit-micro, 6x.30mm, scan at 270nm.
   b) **For Identification:**
      Record spectra between 190 to 400 nm
RESULT OF SCAN HPTLC

%Area of chemical constituents in alcoholic extract of *Yashtimadhu* (scanned at 254nm).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Position</th>
<th>Start Height</th>
<th>Max Position</th>
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<th>Max %</th>
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<td>0.33Rf</td>
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<td>Glycyrrhizin</td>
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<td>0.57Rf</td>
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<td>0.76Rf</td>
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<td>0.82Rf</td>
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<td>9</td>
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<td>0.92Rf</td>
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<td>8419.0AU</td>
<td>7.90%</td>
<td>Unknown</td>
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Solvent system: n-Butanol : Water : Glacial acetic acid (7:2:1).

Scanning: 254nm.
CT INSTITUTE OF PHARMACEUTICAL SCIENCES  
SHAHPUR CAMPUS, VILLAGE: UDOPUR, PRATAPURA ROAD, JALANDHAR (Pb) 144020  
Contact: 09914504420, 0181-5055127

<table>
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<th>Assigned Substance</th>
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<td>0.78</td>
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<td>15.46 AU</td>
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<td>1</td>
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<td>4</td>
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<td>12.74 AU</td>
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<td>23.18%</td>
<td>0.68 Rf</td>
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<td>3.84</td>
<td>38.81%</td>
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<td>0.18 Rf</td>
<td>17.8 AU</td>
<td>0.71 Rf</td>
<td>18.24 AU</td>
<td>11.5</td>
<td>0.72 Rf</td>
<td>10.54 AU</td>
<td>0.86</td>
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<td>10.3 AU</td>
<td>0.74 Rf</td>
<td>10.24 AU</td>
<td>12.11%</td>
<td>0.88 Rf</td>
<td>12.44 AU</td>
<td>1.07</td>
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<td>1.06 AU</td>
<td>0.65 Rf</td>
<td>11.6 AU</td>
<td>6.77%</td>
<td>0.69 Rf</td>
<td>2.54 AU</td>
<td>0.21</td>
<td>4.21%</td>
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</tbody>
</table>

Dr. A K Sharma  
Director, CTIPS
% Area of chemical constituents in G.A Sample of Yashtimadhu (scanned at 254nm).

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<td>376.2AU</td>
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<td>0.30Rf</td>
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<td><strong>28.07%</strong></td>
<td>0.32Rf</td>
<td>11.8AU</td>
<td>8976.6AU</td>
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<td>Glycyrrhizic Acid</td>
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<td>14909.9AU</td>
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<td>2.5AU</td>
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<td>4.21%</td>
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</table>

DISCUSSION-

As traditionally Yastimashu is used against various gastric ailments and growing interest in alternative and complementary medicines, the importance of screening of the drugs are emphasized by the manufacturers worldwide.

Phytochemical screening\(^7\) is necessary in order to establish the identity, purity, safety and quality of Ayurvedic crude drugs for which HPTLC is a standard tool.

From the above observations it was found that the sample drug contains glycyrrhizin and glycyrrhizic acid in the root of yastimadhu. In the drug sample 19.83% Glycyrrhizin is present at 254 nm band, Rf 0.33 and percentage area 13.48%. The second table shows 28.07% Glycyrrhizic acid found at 254 nm band, Rf 0.33 and percentage area 13.48%.

Yastimadhu is a potent drug with broad spectrum application in Ayurveda therapeutics. In this analytical study it is tried to establish the HPTLC findings of yastimadhu for identification of the raw sample. It is crucial for development of fingerprint of the crude yastimadhu for standardization which in turn will lead to preparation of authentic Ayurvedic medicines. Many studies have shown these glycosides to be efficacious for treating viral and gastric disorders.

Conclusion: -In current era, it has become challenging to find out the quality and standard raw material for manufacturing Ayurvedic medicines. As yastimadhu root is used in many formulations and also used as a single drug for treating different ailments, this scientific study attempted to establish the crude yastimadhu root in...
terms of its HPTLC findings. It is concluded that the yastimadhu root is rich in glycosides glycyrrhizin 19.83% and glycyrrholic acid 28.07% in quantity. These chemicals are fingerprint of the yastimadhu root.

References:


CORRESPONDING AUTHOR

Dr. Indrajit Singh Kushwaha,
Assistant Professor
Department of Dravyaguna
A & U Tibbia College Karol Bagh
Govt. NCT New Delhi
Email id – vindadg@gmail.com

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