



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

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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. *Yastimadhu* 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 possesses 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 emphasis and discussed for 11 times^[5]. Now a day modern scientists are keen 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.



Yastimadhu Plant



Yastimadhu Root

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 procedure. 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 *yastimadu* 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).

Peak	Start Position	Start Height	Max Position	Max Height	Max%	End Position	End Height	Area	Area%	Assigned Substance
1	0.03Rf	0.2AU	0.05Rf	282.7AU	11.33%	0.10Rf	38.3AU	8097.2AU	7.59%	Unknown
2	0.19Rf	120.6AU	0.23Rf	178.5AU	7.16%	0.25Rf	72.3AU	6149.2AU	5.77%	Unknown
3	0.27Rf	188.6AU	0.31Rf	494.8AU	19.83%	0.33Rf	10.8AU	14374.7AU	13.48%	Glycyrrhizin
4	0.35Rf	206.5AU	0.45Rf	262.2AU	10.51%	0.49Rf	87.6AU	22514.3AU	21.12%	Unknown
5	0.50Rf	184.7AU	0.56Rf	338.0AU	13.54%	0.57Rf	36.8AU	12073.4AU	11.32%	Unknown
6	0.57Rf	336.9AU	0.60Rf	400.3AU	16.04%	0.67Rf	06.4AU	20934.1AU	19.63%	Unknown
7	0.67Rf	107.3AU	0.72Rf	183.4AU	7.35%	0.73Rf	79.2AU	7150.6AU	6.71%	Unknown
8	0.74Rf	179.6AU	0.76Rf	185.5AU	7.44%	0.82Rf	0.5AU	6913.0AU	6.48%	Unknown
9	0.83Rf	0.4AU	0.88Rf	169.6AU	6.80%	0.92Rf	29.6AU	8419.0AU	7.90%	Unknown

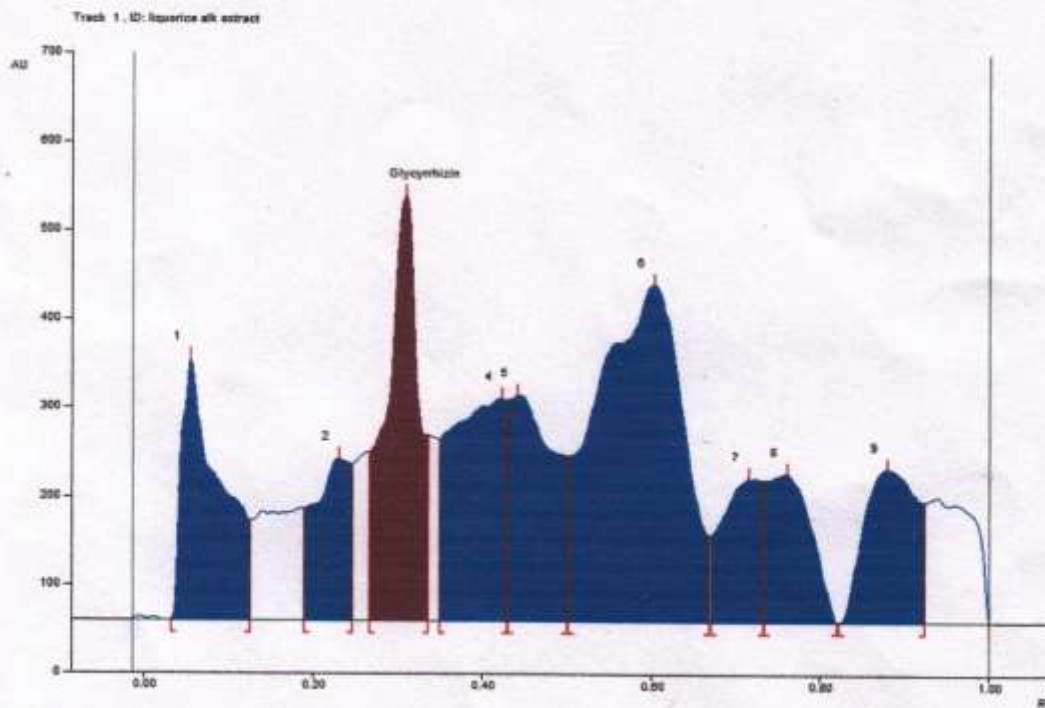
CT INSTITUTE OF PHARMACEUTICAL SCIENCES

SHAHPUR CAMPUS, VILLAGE: UDOPUR, PRATAPURA ROAD, JALANDHAR (Pb) 144020

Contact: 09914504420, 0181-5055127

Solvent system: n-Butanol : Water : Glacial acetic acid (7 :2 :1).

Scanning: 254nm.



Track 1, ID: Squaric alk extract

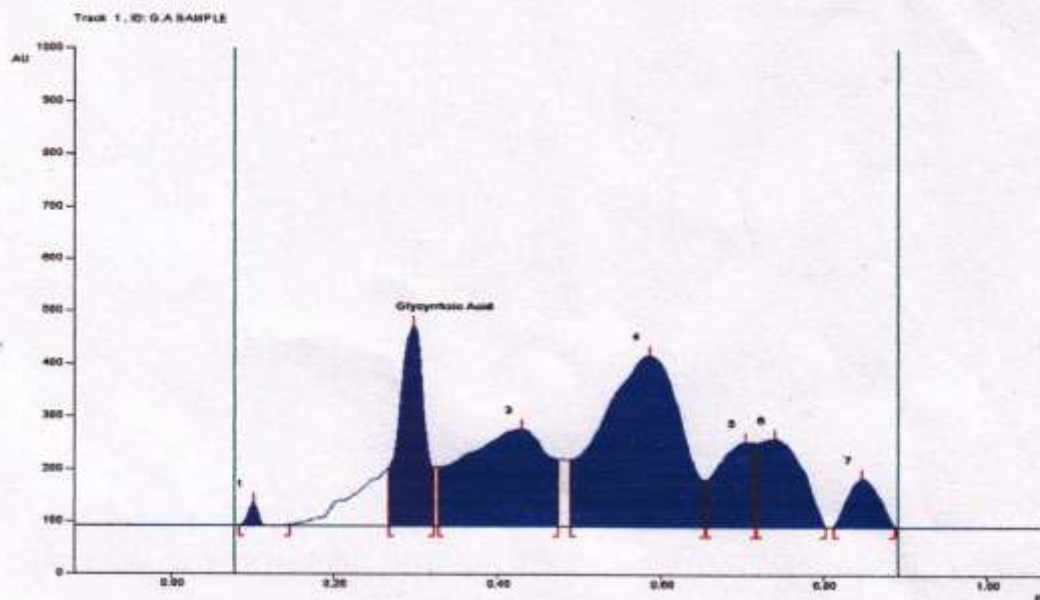
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.05 Rf	2.2 AU	0.05 Rf	282.7 AU	11.53 %	0.16 Rf	36.3 AU	887.2 AU	7.06 %	unknown *
2	0.19 Rf	129.9 AU	0.23 Rf	178.5 AU	7.16 %	0.25 Rf	72.3 AU	8149.2 AU	5.77 %	unknown *
3	0.27 Rf	188.6 AU	0.31 Rf	494.8 AU	19.83 %	0.33 Rf	18.8 AU	14374.7 AU	13.48 %	Glycyrrhizin
4	0.35 Rf	256.3 AU	0.45 Rf	262.2 AU	18.51 %	0.49 Rf	87.6 AU	22514.3 AU	21.12 %	unknown *
5	0.52 Rf	194.7 AU	0.58 Rf	330.0 AU	13.54 %	0.57 Rf	38.9 AU	12673.4 AU	11.32 %	unknown *
6	0.57 Rf	330.0 AU	0.60 Rf	480.3 AU	18.64 %	0.67 Rf	86.4 AU	20924.1 AU	19.63 %	unknown *
7	0.67 Rf	187.3 AU	0.72 Rf	183.4 AU	7.35 %	0.73 Rf	79.2 AU	7158.6 AU	6.71 %	unknown *
8	0.74 Rf	179.6 AU	0.76 Rf	185.6 AU	7.44 %	0.82 Rf	8.5 AU	8913.8 AU	8.48 %	unknown *
9	0.83 Rf	0.4 AU	0.83 Rf	109.6 AU	6.00 %	0.82 Rf	23.6 AU	8419.6 AU	7.86 %	unknown *

Sub
26/3/14
Dr. A.K Sharma
Director, CTIPS

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Contact: 09914504420, 0181-5055127



Track 1, ID: G.A SAMPLE

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1m	0.06 RT	0.2 AU	0.10 RT	42.4 AU	3.13 %	0.15 RT	2.3 AU	376.2 AU	0.59 %	1
2m	0.27 RT	111.1 AU	0.30 RT	300.5 AU	28.07 %	0.32 RT	11.8 AU	8976.6 AU	13.98 %	Glycyrrhizic Acid
3	0.33 RT	112.8 AU	0.43 RT	165.4 AU	13.67 %	0.48 RT	26.2 AU	14809.9 AU	23.22 %	3
4	0.48 RT	127.4 AU	0.59 RT	325.2 AU	23.09 %	0.66 RT	89.2 AU	24657.6 AU	38.41 %	4
5	0.66 RT	89.6 AU	0.71 RT	162.0 AU	11.95 %	0.72 RT	60.5 AU	5599.1 AU	8.72 %	5
6	0.72 RT	160.3 AU	0.74 RT	168.2 AU	12.41 %	0.89 RT	1.2 AU	6979.2 AU	10.87 %	6
7	0.81 RT	1.0 AU	0.85 RT	91.8 AU	6.77 %	0.89 RT	2.5 AU	2703.1 AU	4.21 %	7

Sub
26/3/14
Dr. A K Sharma
Director, CTIPS

%Area of chemical constituents in G.A Sample of *Yashtimadhu* (scanned at 254nm).

Peak	Start Position	Start Height	Max Position	Max Height	Max%	End Position	End Height	Area	Area%	Assigned Substance
1	0.08Rf	0.2AU	0.10Rf	42.2AU	3.13%	0.15Rf	2.3AU	376.2AU	0.59%	Unknown
2	0.27Rf	111.1AU	0.30Rf	380.5AU	28.07%	0.32Rf	11.8AU	8976.6AU	13.98%	Glycyrrhizic Acid
3	0.33Rf	112.8AU	0.43Rf	185.4AU	13.67%	0.48Rf	26.2AU	14909.9AU	23.22%	Unknown
4	0.49Rf	127.4AU	0.59Rf	325.2AU	23.99%	0.66Rf	89.2AU	24657.6AU	38.41%	Unknown
5	0.66Rf	89.6AU	0.71Rf	162.0AU	11.95%	0.72Rf	60.5AU	5599.1AU	8.72%	Unknown
6	0.72Rf	160.3AU	0.74Rf	168.2AU	12.41%	0.80Rf	1.2AU	6979.2AU	10.87%	Unknown
7	0.81Rf	1.0AU	0.85Rf	91.8AU	6.77%	0.89Rf	2.5AU	27.3.1AU	4.21%	Unknown

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%.

Yastimaddhu 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 glycyrrhic acid 28.07% in quantity. These chemicals are fingerprint of the *yastimadhu* root.

References:

1. Ayurvedic pharmacology and therapeutic uses of medicinal plants, author- Vaidya V. M. Gogte, reprint 2009, publisher- Chaukhambha publications Ansari road, Daryaganj, New Delhi.
2. Guduchyadiverga, page no.-41, Dhanawantari Nighantu, Acharya Piyavrat Sharma, 4th edition, 2005, publisher- Chaukhambha Orientalia, Varanasi, 2005.
3. Kaidev Nighantu, page no.-22, Aushadhiverga, Acharya Priyavrat Sharma, First edition, publisher- Chaukhambha Orientalia, Varanasi, 1979.
4. Raj Nighantu, Pandit Narahari edited by Indradev Tripathi, First edition, Pipalyadiverga, page no.-164, publisher- Krishna das Academy, Varanasi, 1982.
5. Acharya Agnivesha, Charaka Smahita, Edited by Brahmananda Tripathi, Sutrasthan, 4/41, 3rd Edition, Chaukhambha Surbharti Prakashan, Varanasi, 1994.
6. Acharya Bhava Mishra, Bhavaprakasha Nighantu, Commentary by K.C. Chuneekar, Karpuradivarga, Pp-186, 5th Edition, Chaukhambha Sanskrit Sansthan, Varanasi, 1979.
7. Behera Banshidhar, Yadav Deepmala and Rath Sudipt, Preliminary Phytochemical Screening and Pharmacognostical study of *Citrullus colocynthis* (Linn.) Journal of Drug Research 2013; 2(1): 18-27

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Source of support: Nil
Conflict of interest: None Declared