Research Article

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STANDARDIZATION OF JATYADI GHRITA BY HPTLC METHOD

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ABSTRACT
Standardization of herbal formulation is essential in order to asses the quality of drugs for therapeutic value. According to an estimate of world Health Organization (WHO) nearly 80% of population of developing countries relies on traditional medicine. Jatyadi Ghrita was found in authentic book like Ayurvedic formulary of India and used as a topical product prescribed for the treatment of all kind of wounds of the body. It is a polyherbal preparation containing eleven ingredients. In this research paper an attempt has been made to develop standardization method for some of the ingredients of Jatyadi Ghrita. Quantitative estimation and stability was done by HPTLC methods using Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and kutkin as markers which are major constituents of formulation. A standard laboratory reference sample of Jatyadi Ghrita and four marketed samples were evaluated as per methods. Data has been provided to demonstrate applicability of the methods to standardization of Jatyadi Ghrita.
Keywords: Standardization, Jatyadi Ghrita, HPTLC, Stability study, Traditional medicine.

INTRODUCTION

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani.The need of quality control for those medicine is due to the fact that the preparation of drug according to the ancient method has been reduced due to commercialization of Ayurvedic pharmacy during past era.[1]The concept of quality in those days was based on physical aspects of the plant materials such as identification, colour, odour, size, Age etc. Today there are addition to physical test and identification i.e.chemical composition. The Government of India has adopted the "Fingerprint"approach[2] for botanicals because it supports the traditional concept and is easy to practice at different levels of sophistication. Jatyadi ghrita (JG) is a medicated ghee formulation popularly used in the treatment of various topical wounds. Jatyadi Ghrita, a polyherbal preparation containing eleven ingredients, was found in authentic book. It contains one part each of drug. The formulation describes the presence of *Glycyrrhiza glabra, Jasmine grandiflorum, Pongamia pinnata, Curcuma longa, Berberis aristata and Picrorhiza kurroa,* the principal constituents of which are Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin respectively[3-8]. Separate HPTLC methods were available for analysis of the same constituents.

MATERIALS AND METHODS

All the plants, viz. *Jasmine grandiflorum* Linn. (leaf), *Azadirachta indica* A.Juss. (leaf), *Berberis aristata* D.C. (stem), *Curcuma longa* Linn. (rhizome), *Picrorhiza kurroa* Royle. (root), *Trichosanthes dioica* Roxb. (leaf), *Pongamia pinnata* Linn. (seed), *Hemidesmus indicus* Linn. (root), *Glycyrrhiza glabra* Linn. (root), *Rubia cordifolia* Linn. (root) and *Vetiveria zizanioids* Linn. (root) were collected from the local crude drug market, Chinchwad, Pune and all these plants materials were authenticated by Botany Group, Plant Science Division, Agharkar Research Institute,Pune. (No.3/187/2010/Adm. 367).

Preparation of Jatyadi ghrita

In laboratory, formulation of Jatyadi ghrita was prepared as per Ayurvedic Formulary of India. All the plant parts, viz. *Jasminum grandiflorum, Azadirachta indica, Berberis aristata, Curcuma longa, Picrorrhiza kurroa, Trichosanthes dioica, Pongamia pinnata, Hemidesmus indicus, Glycyrrhiza glabra, Rubia cordifolia,* and *Vetiveria zizanioids* were dried in oven at 45 ^{0}C until they were free from moisture. They were then powdered and sieved through 60# and stored in air tight container for further use. All the drugs were taken in the equal amount that is 1.47 gm for 100gm. Decoction of each drug and 1.47 gm of copper sulphate in 10 ml water were mixed together and passed through muslin cloth. Bees wax 1.47 gm and 76.8 gm Cow's ghee was heated on water bath at100^{0}C. The decoction and copper sulphate solution was mixed it in melted condition with propeller mixer at 100 RPM for 15 min.

A reference sample of Jatyadi Ghrita (JGL) prepared in the laboratory and four marketed formulations JGAV, JGA, JGR and JGN were chosen for the standardization.

Instruments

HPTLC analysis of the formulation was performed by using CAMAG TLC scanner (Scanner_170422, Anchrome), with different chromatographic conditions for different drugs. Mobile phase was established for each drug for best resolution and compared with standard. Six components of the Jatyadi ghrita were evaluated i.e. glycyrrhetinic acid, ursolic acid, karanjin, curcumine, berberine and kutkin which are also major constituents of formulations. All components showed good resolution for each drug in the formulation.

HPTLC analysis was performed with different chromatographic conditions for different constituents as mentioned in Table 1.

Preparation of standard solutions

Stock solutions of all markers prepared separately in methanol. Final concentration was made up by further diluting with methanol which was mentioned in Table 2.

Preparation of sample solutions

Accurately weighed 5 gm formulations were extracted with equal amount of hexane and methanol (20 ml each) by means of separating funnel. It was shaken vigorously and allowed to stand for 5 min for separating the two layers. Methanolic layer was again treated with 10 ml hexane till it was free from fat. Hexane layers were discarded. The volume was made with methanol up to 25 ml by using volumetric flask and filtered through 0.22 micron filter paper. This preparation was used as stock solution. Final concentration was made up by further diluting with methanol which was mentioned in Table 2. Comparison was done between reference laboratory formulation (JGL) and four marketed formulations JGAV, JGA, JGR and JGN.

Table 1: Different Chromatographic Conditions[8-12]

Chromatographic Conditions	Glycyrrhetinic acid	Ursolic acid	Karanjin	Curcumin	Berberine	Kutkin
Stationary phase	TLC plate, silica F_{254}	TLC plate, silica F_{254}	TLC plate, silica F_{254}	TLC plate, silica F_{254}	TLC plate, silica F_{254}	TLC plate, silica F_{254}
Mobile phase	A] Chloroform: acetone (9:1) B] Chloroform :diethyl ether: formic acid (80:15:1)	Toluene: ethyl acetate (7:3)	Toluene: ethyl acetate (7:3)	Toluene: ethyl acetate: formic acid (5:1.5:0.5)	n-propanol: formic acid: water (90:1:9)	Ethyl acetate: methanol: glacial acetic acid (18:5:0.2)
Derivatization	-	Sprayed with 10% H_2SO_4 solution in methanol and 120^0C for 365 nm and visible light.	-	-	-	Sprayed with anisaldehyde H_2SO_4 reagent and saw Under UV at 254 nm.
Scanning	254nm	520nm	260nm	254nm	254nm	280nm
Rf value	0.25	0.41	0.63	0.44	0.28	0.64

Table 2: Concentration of Standard and Sample solutions

Analysed Constituents	Glycyrrhetinic acid	Ursolic acid	Karanjin	Curcumin	Berberine	Kutkin
Standard solution (µg/ml)	0.6	6	0.6	0.6	0.6	0.6
Test solution (µg/ml)	6	0.12	6	6	6	30

Table 3: Percent concentration of Constituents

Constituents	Formulations : Percentage Concentration				
	JGL	JGAV	JGA	JGR	JGN
Glycyrrhetinic acid	2.05	1.48	1.4	1.56	1.63
Ursolic acid	1.5	1.52	1.4	1.42	1.4
Karanjin	1.27	1.27	1.25	1.19	1.26
Curcumin	1	1.06	1.05	0.43	1.16
Berberine	1.21	1.2	1.16	0.7	1.21
kutkin	0.91	0.87	0.50	0.55	0.61

Table 4: Stability data

Parameters	Time Period		
	Initial	Third month	Six month
Concentration of Glycyrrhetinic acid (%)	2.05	2.03	2.01
Concentration of Ursolic acid (%)	1.5	1.46	1.45
Concentration of Karanjin (%)	1.27	1.25	1.23
Concentration of Curcumin (%)	1	0.98	0.97
Concentration of Berberine (%)	1.21	1.20	1.18
Concentration of Kutkin (%)	0.91	0.90	0.88
pH	4.7	4.7	4.7
color	Yellowish green	Yellowish green	Yellowish green
Viscosity (Pascal second)	8000	8000	8000

Stability study

Stability of Ayurvedic formulation can be monitored by chromatographic analysis and developing a HPTLC chemo profile for the product. In the present studies stability of the herbal formulation was monitored for six month after packaging of formulation in tubes and storing them at 30^0C of temperature and 65% humidity. Samples were drawn at the time interval of three and six months. The Samples were tested for its stability in terms of its drug content and physicochemical parameters. [12, 13]

RESULTS AND DISCUSSION

HPTLC finger printing profile

HPTLC study of methanolic extracts of the in-house formulation and marketed formulations were carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in all the formulations. Inject the prepared standard and sample solutions and quantitative analysis was carried out by comparison with respective peak areas. The HPTLC fingerprint profiles of the formulations are presented in Figure 1-6.

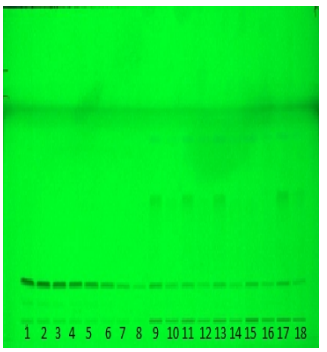

Figure 1: HPTLC fingerprint profile of Glycyrrhetinic acid and Jatyadi formulations
Track 1-8: Standard Glycyrrhetinic acid
Track 9 &10: Formulation JGL
Track 11&12: Formulation JGAV
Track 13&14: Formulation JGA
Track 15&16: Formulation JGR
Track 17&18: Formulation JGN

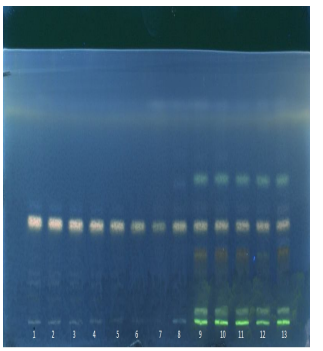

Figure 2: HPTLC fingerprint profile of Ursolic acid and Jatyadi formulations
Track 1-8: Standard Ursolic acid
Track 9: Formulation JGL
Track 10: Formulation JGAV
Track 11: Formulation JGA
Track 12: Formulation JGR
Track 13: Formulation JGN

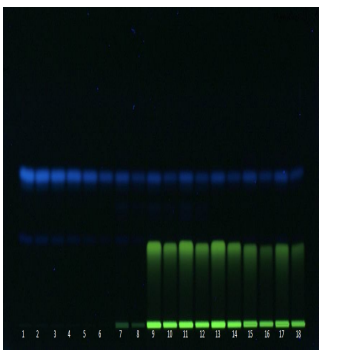

Figure 3: HPTLC fingerprint profile of Karanjin and Jatyadi formulations
Track 1-8: Standard Karanjin
Track 9 &10: Formulation JGL
Track 11&12: Formulation JGAV
Track 13&14: Formulation JGA
Track 15&16: Formulation JGR
Track 17&18: Formulation JGN

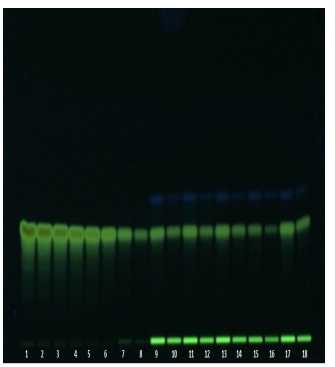

Figure 4: HPTLC fingerprint profile of Curcumin and Jatyadi formulations
Track 1-8: Standard Curcumin.
Track 9 &10: Formulation JGL
Track 11&12: Formulation JGAV
Track 13&14: Formulation JGA
Track 15&16: Formulation JGR
Track 17&18: Formulation JGN

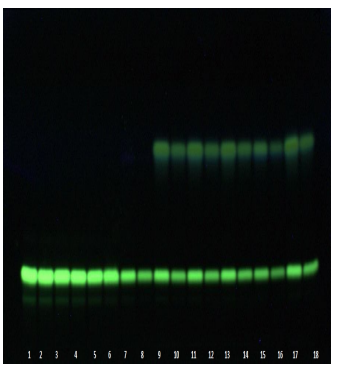

Figure 5: HPTLC fingerprints profile of Berberine and Jatyadi formulations.
Track 1-8: Standard Berberine
Track 9 &10: Formulation JGL
Track 11&12: Formulation JGAV
Track 13&14: Formulation JGA
Track 15&16: Formulation JGR
Track 17&18: Formulation JGN

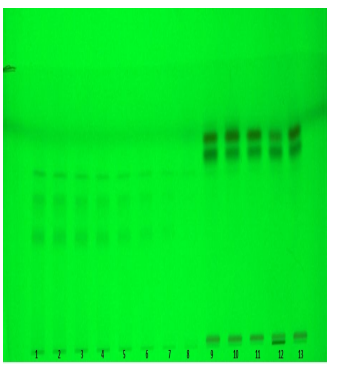

Figure 6: HPTLC fingerprint profile of Kutkin and Jatyadi formulations
Track 1-8: Standard Kutkin
Track 9: Formulation JGL
Track 10: Formulation JGAV
Track 11: Formulation JGA
Track 12: Formulation JGR
Track 13: Formulation JGN

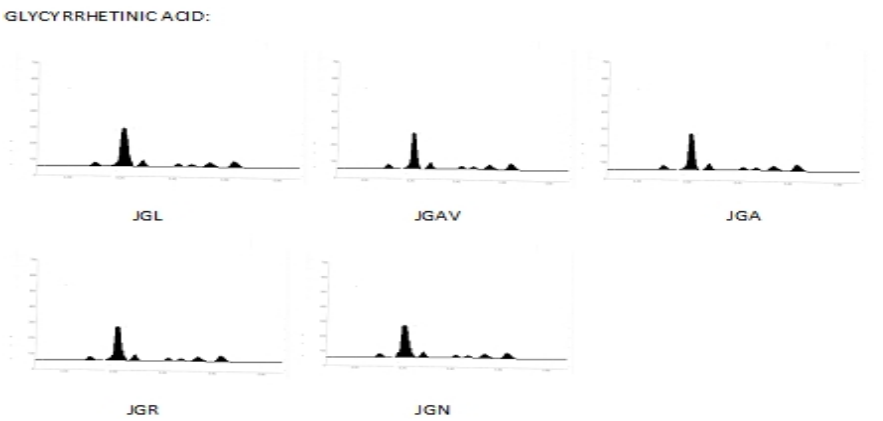

Figure 7: HPTLC of Glycyrrhetinic acid in Formulations

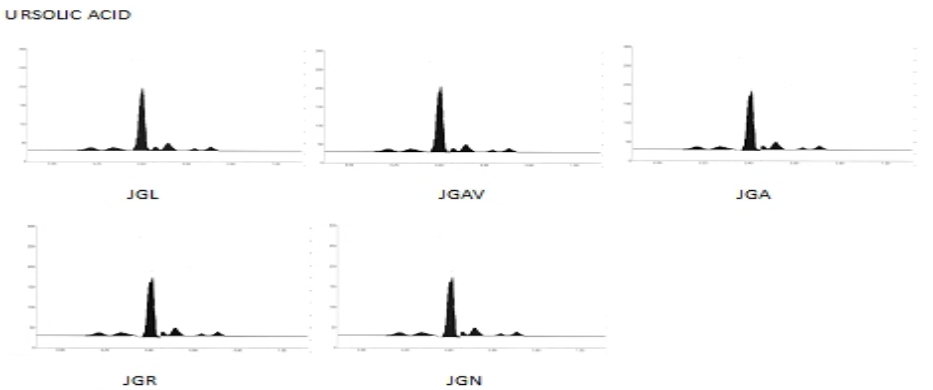

Figure 8: HPTLC of Ursolic acid in Formulations

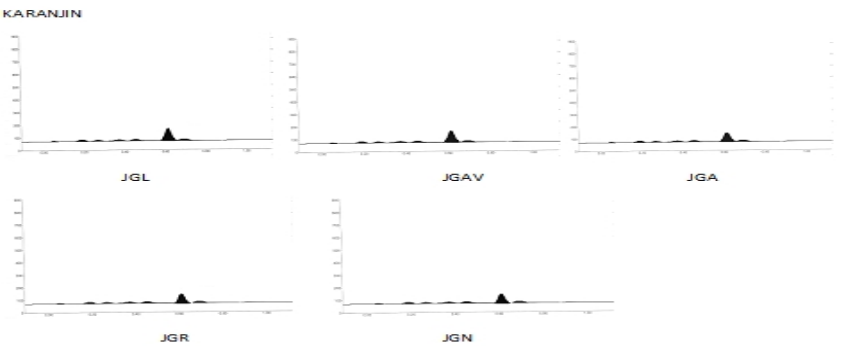

Figure 9: HPTLC of Karanjin in Formulations

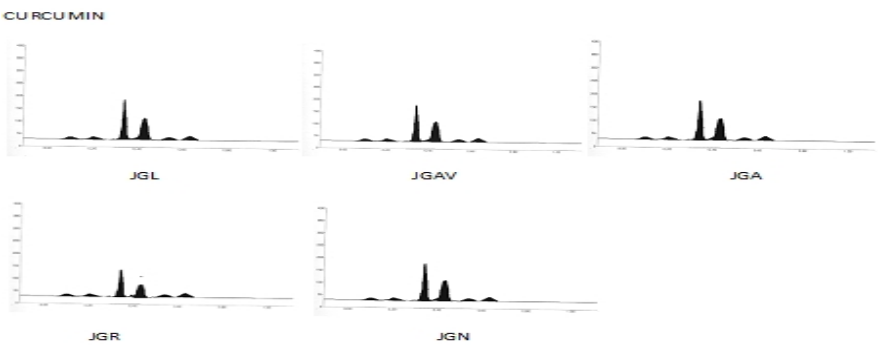

Figure 10: HPTLC of Curcumin in Formulations

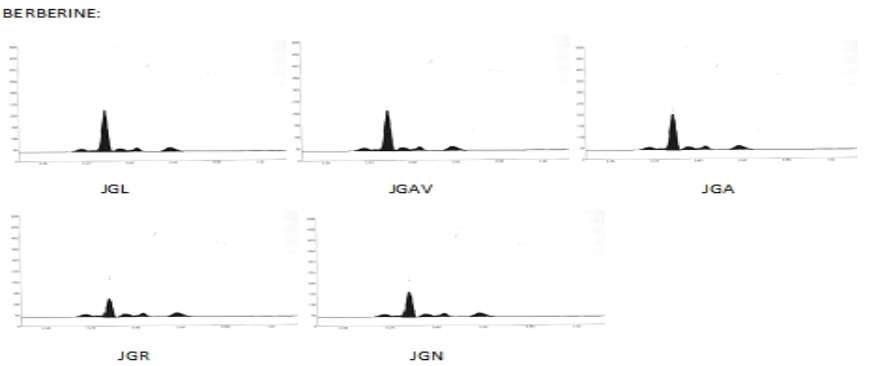

Figure 11: HPTLC of Berberine in Formulations

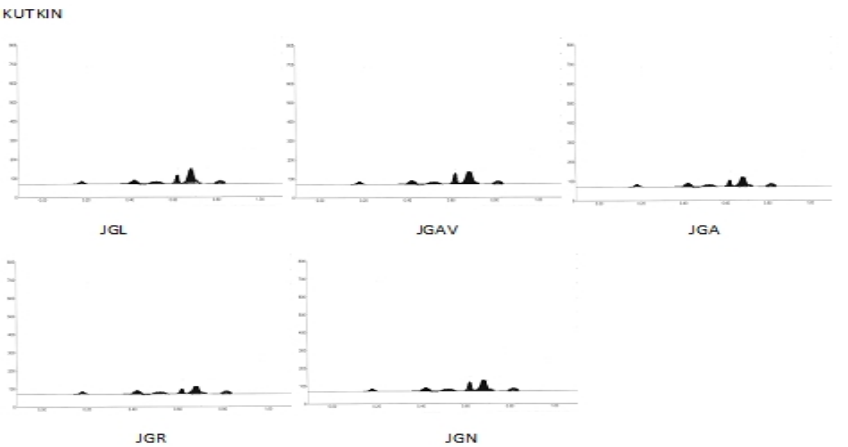

Figure 12: HPTLC of Kutkin in Formulations

The percentage of concentration for each constituents were found in the reference laboratory formulation (JGL) and four marketed formulations JGAV, JGA, JGR and JGN mentioned in Table 3.

Stability study

For the stability study formulations were drawn at the time interval of three and six months. The samples were tested for its stability in terms of its drug content and physicochemical parameters. This was shown in the Table 4.

HPTLC study of formulations was done to calculate the presence of ingredient. The marker compound Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin were estimated by HPTLC in Jatyadi ghrita sample R_f 0.25 corresponding to Glycyrrhetinic acid, 0.41 corresponding to Ursolic acid, 0.63 corresponding to Karanjin, 0.44 corresponding to Curcumin, 0.28 corresponding to Berberine and 0.64 corresponding to Kutkin, these are visible in the formulations. HPTLC fingerprint profile of the Jatyadi ghrita formulations are depicted in figure 1-6 indicates the presence of all the ingredients in proportional quantity in the formulations. This also confirms the band-to-band consistency of the finished product. From the stability study there were no significant changes in the physicochemical parameters and concentration of Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin in the formulations. It shows that the formulation was stable.

Ayurvedic medicine Jatyadi ghrita has been standardized by intervention of modern scientific quality control measures described in classical texts. HPTLC fingerprint profiles together may be used for quality evaluation and the standardization of compound formulations.

CONCLUSION

It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The use of markers ensures the concentration and ratio of components in the herbal mixture. This study was also focused on quantitative estimation of glycyrrhetinic acid, ursolic acid, Karanjin, curcumine, berberine and kutkin which are major constituents of Jatyadi Ghrita by using modern methods of analysis. Stability study of formulation shows the ability of formulation to remain with its physical chemical, pharmacological, microbiological, therapeutic and toxicological property.

Abbreviations

WHO-World Health Organization
HPTLC-High Performance Thin layer Chromatography.
JG-Jatyadi Ghrita
JGL- Jatyadi Ghrita Laboratory preparation
JGAV, JGA, JGR, JGN-Jatyadi Ghrita Marketed Preparations.
TLC- Thin Layer Chromatography

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