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PHARMACEUTICO-ANALYTICAL STUDY AND QUALITY STANDARD DETECTION OF JATYADI GHRITA BY HPTLC METHOD

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ABSTRACT

In the era of commercialization quality control and standardization of herbal formulation is essential in order to assess the quality of drugs for therapeutic value. *Jatyadi Ghrita* is a ghee based Ayurvedic formulation useful for treatment of all kind of wounds by external application found in classical texts of *Ayurveda*. In this study an attempt has been made to develop standard for *Jatyadi Ghrita* by HPTLC method. It was prepared by standard laboratory reference of Ayurvedic formulary of India in three batches in RS & BK Laboratory NIA, Jaipur. The physicochemical tests performed on different samples of *Jatyadi Ghrita* such as acid value, saponification value, Total fatty matter, melting point, viscosity etc. Further standardization and comparative evaluation of laboratory sample and marketed sample done by instrument analysis performed on HPTLC finger printing profile. Data has been provided to demonstrate applicability of the methods to standardization of *Jatyadi Ghrita.*

Keywords: *Jatyadi Ghrita,* HPTLC Fingerprint Profile, Traditional medicine, Quality standard

INTRODUCTION

The traditional systems of medicine evolved over centuries had been responsible for safeguarding healthcare of the world until the advent of allopathic system of medicine. As the later system has used the knowledge of modern biology and chemistry, for both discovery and treatment. This system got fast acceptability among

the users and now it occupies predominant space in the area of healthcare. In spite of this, the contribution of the traditional preparations, which are normally poly-herbal, is increasing because of the general impression that these products are safe; while the single-molecule based modern drugs used in allopathic system can have severe adverse effects.[1] Although Commercialization started disadvantages came forward peoples become doubtful regarding quality and genuinity of drugs what they had prescribed by the physician and what they are going to purchase, enquires scientific reasoning leads to introduction of quality control and standardization. The need of quality control for Ayurveda, Sidhha and Unani (ASU) drug is because the preparation of drug according to the ancient method has been reduced due to commercialization of Ayurvedic pharmacy during past era.[2]

It should be renowned that herbal drug standardization is not new in Ayurveda. In the classics, it is point out in a codified manner i.e. *Grahya Lakshana*, Method of collection etc. for raw drug, *Siddhi Lakshanas* for final product and for medicament, *Alpamatram, Mahavegam, Bahudoshaharam* etc. properties are mentioned in *Ayurvedic* classics. Beside this, so many fundamental standards regarding drugs are available in our classics, which are primitive and limited to the time need of that stage. The concept of quality in those days was build up based on physical aspects of the plant materials such as identification, color, odor, 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 for botanicals because it supports the traditional concept and is easy to practice at different levels of sophistication.[3]

Jatyadi Ghrita is a ghee-based herbo-mineral formulation useful for wounds, painful ulcers, insect bite wounds, wounds caused by heat or fire and deep wounds by external application as per the classical texts of Ayurveda.[4,5,6,7,8] As it is a popular classical formulation there is a need to standardize this formulation. *Jatyadi ghrita* marketed formulation J G M was chosen for analytical standardization and three *Jatyadi Ghrita* samples JGL1, JGL2, JGL3 were prepared in laboratory by same SOP for pharmaceutico-analytical standardization. The physicochemical tests performed on different samples of *Jatyadi Ghrita* such as specific gravity, acid value, saponification value, Total fatty matter, melting point, viscosity etc. to know their physico-chemical properties. Further standardization by instrument analysis performed on HPTLC for finger printing profile. Hence a comparative study of market sample and laboratory sample was done by sophisticated techniques such as HPTLC method to know the need for standardization of Ayurvedic formulations.

Aim and Objectives –

Preparation of *Jatyadi Ghrita* in three batches to standardization of pharmaceutical process. To evaluate physico-chemical parameters of the above samples. For further standardization Instrument analysis with HPTLC finger printing profile.

Material & Methods:

One *Jatyadi Ghrita* formulation of Nagarjuna Ayurveda (batch no. PD8E) was purchased from local market of Chomu, Jaipur, Rajasthan and coded as JGM.

Pharmaceutical Study: All the raw materials were procured from the pharmacy and authenticated by Drug testing laboratory in Dept. *Rasashastra* and *Bhaishajya Kalpana*, National Institute of Ayurveda, Jaipur. Ingredients used in preparation of *Jatyadi Ghrita* are tabulated below.

Methods: *Jatyadi Ghrita* was prepared with proper method and standards as per the reference of AFI[9] in 3 batches in the Dept. of *Rasashastra* and *Bhaishajya kalpana* laboratory, National institute of Ayurveda, Jaipur. Kalka was prepared by 2-12 drugs after cleaned that made into powder separately and pass through 250µm I.S. sieve then added water till the mixture become *Kalka* form. The quantity of fresh leaves was taken just double from other dry *Kalka dravya's*.[10] *Kalka Dravyas* were transferred to the wet grinder and grind with sufficient quantity of water to prepare homogeneous blend. *Ghrita Paka* was done by Murchhita *Goghrita*,[11] *Kalka* and *Jala* on mild heat {between 50^0 and 90^0} till *Sneha Siddhi Lakshana* appeared with intermittent stirring on 1st and 2nd day and continuous stirring on 3rd day. It was filtered through muslin and *Prakshepa Dravya* were added to it. *Shodhita Tuttha* in

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10 ml water were mixed together and passed through muslin cloth. Bees wax was heated with some quantity of ghee on water bath at 100^0C. The copper sulphate solution was mixed it in melted condition with propeller mixer at 100 RPM for 15 min. and remaining *Ghrita* was added slowly with continuous stirring. Above same procedure was conducted in 3 samples of *Jatyadi Ghrita*.

S. N.	Name of the drugs	Part of use	Quantity	
1.	*Murcchhita Goghruta*	Cow's ghee	768 g	1 part- Sneha
2.	*Jati Patra* {*Jasminum grandiflorum*}	Fresh Leaf	35 g	¼ part
3.	*Nimba Patra* {*Azadirachta indica*}	Fresh Leaf	35 g	*Kalka Dravya*
4.	*Patol Patra* {*Trichosanthes dioica*}	Dried Leaf	17.45g	
5.	*Katuka* {*Picrorhiza kurroa*}	Rhizome /Root	17.45g	
6.	*Daruharidra* {*Berberis aristata*}	Stem	17.45g	
7.	*Haridra* {*Curcuma longa*}	Rhizome	17.45g	
8.	*Sariva Hemidesmus indicus*}	Root	17.45g	
9.	*Manjishtha* {*Rubia cordifolia*}	Root	17.45g	
10.	*Abhaya* (*Ushira*) *Vetiveria zizanioides*	Root	17.45g	
11.	*Madhuyashthi* {*Glycyrrhiza glabra*}	Root	17.45g	*Kalka*
12.	*Karanja Beeja* {*Pongamia pinnata*}	Seeds	17.45g	
13.	*Jala*	Potable water	3.07 l kg	4 part-*Drava*
14.	*Siktha*	Bee's wax	17.45g	*Prakshepa Dravya*
15.	*Tuttha* {Copper sulphate}	$CuSo_4$	17.45g	,,

Analytical Study: The physicochemical analysis of *Jatyadi Ghrita* was conducted on the basis of parameters laid down by API at Ayushraj Enterprises Pvt. Ltd. Dehami Begas Road, Mansinghpura Jaipur and S.R. LABS Haldighati Marg, Pratap Nagar Jaipur. such as Organoleptic characters, Rancidity, Specific gravity, Refractive index, Viscosity, Iodine value, Acid value, Peroxide value, Saponification value etc. as per standard procedure of *Ayurvedic* Pharmacopoeia of India.[12]

HPTLC Fingerprint profile: HPTLC performed by TLC apparatus CAMAGE, Switzerland at Ayushraj Enterprises Pvt. Ltd. Dehami Begas Road, Mansinghpura Jaipur Instrument consists of Linomat 5 applicator, Scanner 4, photographic chamber and visualization chamber. The procedure was performed as per the reference of API.[13]

Conditions: Stationary phase: Pre-coated silica gel 60 F_{254} aluminum plates

Mobile phase: Toluene: Ethyl acetate: formic acid [6:3.5:0.5]

Chamber Saturation Time: 20 mins.

Test Solution: 2 g of every sample of JG were separately extracted by cold maceration in methanol and then filter the liquid extract and made the volume up to 50 ml with methanol.

Derivatizing Reagent: Anisaldehyde Sulphuric acid

Procedure: Previously dried TLC plate were taken and fix dimension at X position and mark from base with help of pencil at 10 mm and 90 mm and also left 20 mm from both sides of plate. The test sample solution was applied 6 µl in the form of bands with the programming of Linomats applicator. Then solvent was allowed to be evaporated and place the plate in the saturated tank, possibly vertical and so that spots or bands are above the level of mobile phase. After closing the tank and allowed it to stand at room temperature until mobile phase ascended to the marked line. Remove the plate and dry and visualize as in UV light at 254 nm. The photographs were taken of developed and derivatized TLC plate at 254nm, 366nm &white light. Process was carried on derivatization of plate by dipping method in anisaldehyde reagent. Dried over 100^0C for 15min and visualize in day light. The scanning Programme for

completely dried plate was prepared at different wave-length of 550nm, 625nm and 360 nm. Comparative evaluation of the scanning parameter (Densitogram) is performed by final analysis report.

Observation & Results: These are drawn in tables as below-

Table 1: Showing organoleptic characteristics of laboratory JG samples

Sample	Color	Odor	Appearance	Clarity
Murchita Ghrita	Yellowish green	Characteristic	Granular, oily	Opaque
JGL1	Greenish yellow	Characteristic	Granular, oily	Opaque
JGL2	Greenish yellow	Characteristic	Granular, oily	Opaque
JGL3	Greenish yellow	Characteristic	Granular, oily	Opaque
JGM	Greenish yellow	Characteristic	Granular, oily	Opaque

Table 2: Showing the weight loss of all samples of *Jatyadi Ghrita* in *Sneha Paka* Process.

Name of the sample	Initial weight of *Murcchhita* Sneha (g)	Weight of obtained *Jatyadi Ghrita* (g)	Loss of weight (g)	% of loss	Mean % of loss
JGL-1	768 g/870 ml	695.5	72.5	9.4 %	9.26%
JGL-2	768 g/870 ml	699	69	8.9%	
JGL-3	768 g/870 ml	694.6	73.4	9.5 %	

Table 3: Comparative Physiochemical analysis of *Jatyadi Ghrita* (JG) samples

Physio-chemical Parameters	Different *Jatyadi Ghrita* Laboratory Samples			*Jatyadi Ghrita* Marketed sample
	JGL1	JGL2	JGL3	JGM
Acid Value	2.98	2.28	1.91	2.99
Rancidity	Slightly rancid	Slightly rancid	Slightly rancid	Slightly Rancid
Unsaponifiable Matter % (w/w)	0.97	0.88	1.02	0.89
Saponification value	174.35	151.79	161.82	170.35
Total Fatty Matter % (w/w)	88	81.3	84	82.3
Ester Value	171.37	149.51	159.91	163.51
Refractive Index	1.428	1.428	1.428	1.428
Specific Gravity	0.925	0.930	0.916	0.929
Viscosity	47cp (At 40^0C)	-	-	49cp(At 40^0C)
Peroxide Value	32.47	25.94	20.29	26.96
Iodine value	5.08	7.48	4.75	7.52
Melting Point	39^0 C	-	-	40^0C
Heavy Metal Analysis				
Lead	ND	-	-	-
Cadmium	0.038 ppm	-	-	-
Mercury	ND	-	-	-
Arsenic	ND	-	-	-

Table 4: Showing Rf values obtained from HPTLC fingerprint profile of *JG samples*

Scanning Wavelengths	Rf Values of Major Spots	
	JGM	JGL1
At 550 nm	0.31, 0.38, 0.46, 0.59, 0.60, 0.68, 0.76, 0.64	0.13, 0.19, 0.32, 0.39, 0.47, 0.53, 0.59, 0.64, 0.68, 0.78
At 625 nm	0.26, 0.31, 0.37, 0.44, 0.59, 0.64, 0.68, 0.76	0.13, 0.19, 0.27, 0.32, 0.38, 0.47, 0.53, 0.59, 0.64, 0.68, 0.77
At 700 nm	0.26, 0.31, 0.38, 0.58, 0.63, 0.68, 0.76	0.13, 0.19, 0.27, 0.32, 0.36, 0.38, 0.45, 0.59, 0.64, 0.69, 0.79

Table 5: Showing the results of HPTLC of JG marketed & laboratory samples

Sample	550 nm wavelength	625 nm wavelength	700 nm wavelength
JGM	8 spots visualize	9 spots visualize	9 spots visualize
JGL1	11 spots visualize	12 spots visualize	12 spots visualize

The HPTLC Fingerprint profile of *Jatyadi* formulations are presented in figure 1-6

Figure 1: HPTLC Banding pattern at 254 nm of JGM (First 3 tracks from Left to right) and JGL1 (Last 3 tracks)

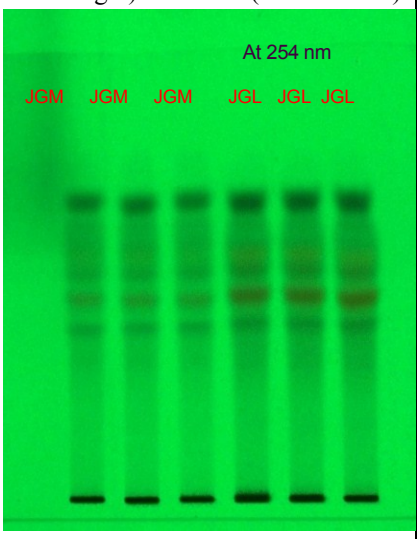


Figure 2: HPTLC Banding pattern at 366 nm of JGM (First 3 tracks from Left to right) and JGL1 (Last 3 tracks)

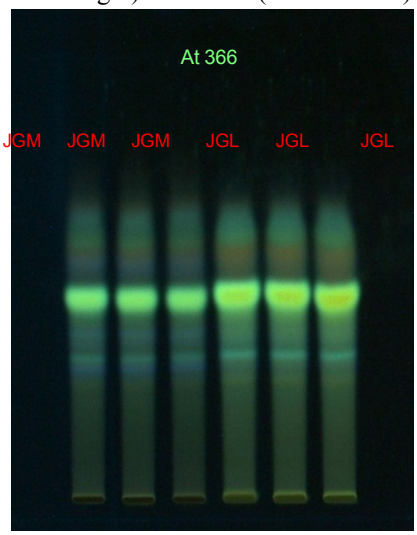


Figure 3: HPTLC Banding pattern at 366 nm of derivatized plate for JGM (First 3 tracks from Left to right) and JGL1 (Last 3 tracks)

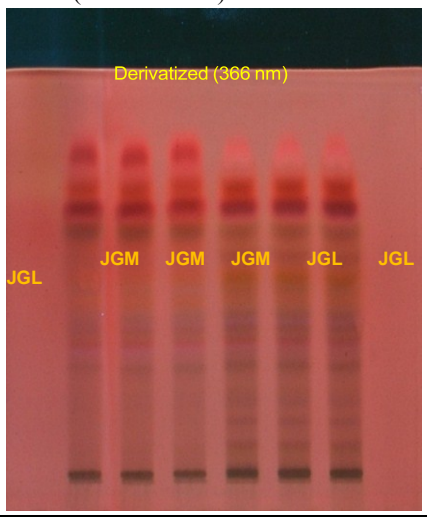


Figure 4: HPTLC Banding pattern at white (First 3 tracks from Left of derivatized plate for JGM

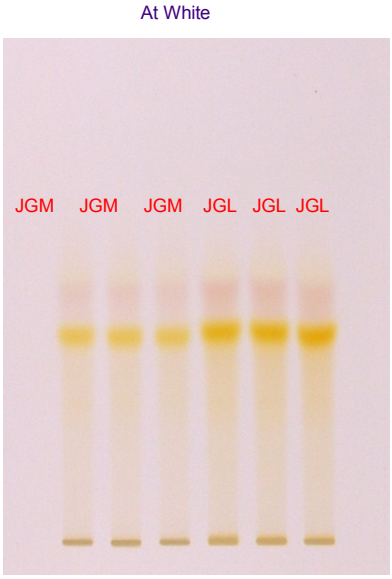
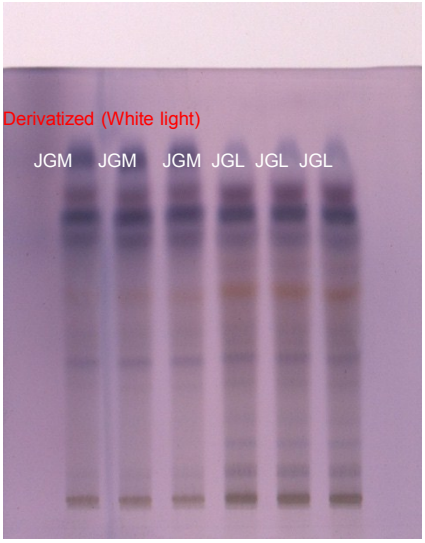


Figure 5: HPTLC Banding pattern at white light of JGM (First 3 tracks to right) and JGL1 (Last 3 tracks from Left to right) and JGL1(Last 3 tracks)



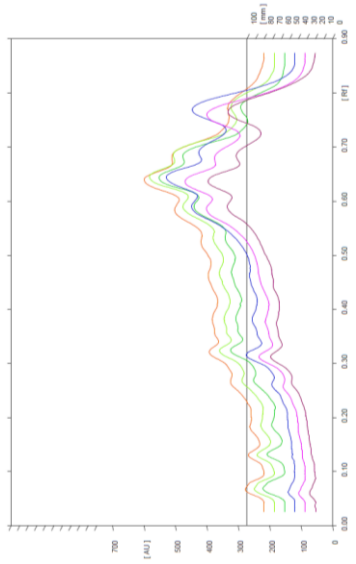


Figure 6: Scanning pattern of all the track showing significant difference between JGM and JGL1 formulations.

DISCUSSION

HPTLC study of methanolic extracts of the formulation *Jatyadi Ghrita's* market sample and laboratory prepared samples were carried out. Inject the prepared and marketed sample solutions and qualitative analysis was

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carried out by comparison with respective peak areas. Samples were analyzed on different wavelengths 550nm, 625nm and 700nm. The scanning data shows different spots visualized get confirms the chemical nature and distribution pattern in specified mobile phase. The HPTLC fingerprint profiles of the formulations are presented in Figure 1-5. It confirms 8-9 distinct spots of marketed *Jatyadi Ghrita* sample and 11-12 distinct spots of laboratory prepared *Jatyadi Ghrita* sample. Figure 6 shows JGM have 8-9 peaks areas and JGL have 11-13 peak areas. HPTLC fingerprint comparison of JGM and JGL was clearly designated the more spots in JGL at lower Rf values which confirm extraction and stability of more secondary metabolites instead of JGM formulation. The Rf values of spots differentiate the samples chemical composition and formulation. Also, the visible pattern and colors of spots at 550nm confirms the samples standardization in visible spectrum. Results of Physico-chemical parameters follows the range of standards laid down by Ayurvedic Pharmacopeia of India for *Jatyadi Ghrita* formulation and heavy metals are also in permissible limits that shows safety and quality of the formulation.

CONCLUSION

Hence, we can conclude HPTLC Fingerprint profiles confirm standard scanning of samples to generate the standardization parameter for quality control purpose. Comparative evaluation of physico-chemical parameters confirmed that *Jatyadi* Laboratory samples and marketed samples are not very much differ in their characterization. Related physico-chemical tests and HPTLC profile clearly shows the more stability of formulation and increased number of secondary metabolite extraction in laboratory sample than marketed sample of *Jatyadi Ghrita*. Possibly the above results denote standardization by sophisticated techniques is need of today.

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