Research Article

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PHARMACEUTICO-ANALYTICAL STUDY OF JATYADI GHRITA AND JATYADI KER TAILA WITH SPECIAL REFERENCE TO THEIR HPTLC FINGERPRINTS PROFILE

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ABSTRACT

Jatyadi Ghrita is a ghee based Ayurvedic formulation useful for sravi (secretory), gambhira (deep), Dushta Vrana (contaminated wound) by external application by shodhana (cleaning) and ropana (healing) as per the classical texts of Ayurveda. Now day's pharmacies are using coconut oil instead of ghrita for preparation of Jatyadi Ker Taila, but remaining contents are same as that of Jatyadighrita as quoted in our Samhitas. Hence a comparative study on Jatyadighrita as per classical text and Jatyadi Ker Taila (modified method by using coconut oil replacing ghrita) to know their physico-chemical properties. The physicochemical tests performed on different samples of Jatyadi Ghrita and Jatyadi Ker taila such as specific gravity, acid value, saponification value, Total fatty matter, melting point, viscosity etc. Further standardization by instrument analysis performed on HPTLC for finger printing profile. Comparative evaluation of above parameters shows that Ker taila (coconut oil) can be used as better substitute of ghrita for the preparation of Jatyadi formulation. Related physicochemical tests and HPTLC profile clearly shows the more stability of formulation and increased number of secondary metabolite extraction in Ker taila than ghrita. Possibly the above results denote best substitution of classical formulation Jatyadi ghrita is Jatyadi Ker taila (by using coconut oil replacing ghrita).

Keywords: Coconut oil, ghrita, HPTLC profile, Jatyadi formulation, Physicochemical.

INTRODUCTION

Pharmaceutical research is aimed at meeting the medical needs of the population for whom appropriate therapeutic remedies are not available at those that are available are unsafe for prophylactic use for various disorders. While meeting medical exist and that the product will command sales and profits proportionate to investments.¹ 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.² Although Commercialization started disadvantages came forward people become doubtful regarding quality and originality 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 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.³ 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 (acceptable qualities), Method of collection etc. for raw drug, Siddhi Lakshanas (signs of end product formation) for final product and for medicament, Alpa matram (required in small dose), Mahavegam (high potency), Bahudoshaharam (broad spectrum/pacifying many doshas) 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" approach⁴ for botanicals because it supports the traditional concept and easy to practice at different levels of sophistication.⁵

Jatyadi Ghrita is a ghee-based 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.⁶⁻⁸ It is a polyherbal preparation containing eleven herbal ingredients.⁹⁻¹¹ Ingredients of Jatyadi Ghrita have been deliberate extensively for their antimicrobial, anti-inflammatory and antiseptic activity that may be responsible for its efficacy in wound healing. But Now a day some pharmacies are using coconut oil instead of ghrita due to abundance of coconut in south India for preparation of Jatyadi Ker Taila (JATYADI KER TAILA) {here Ker means coconut} but remaining contents are same as that of Jatyadi ghrita as quoted in our Samhitas. Despite the fact that it is in large quantities used but nowhere a study conducted before on Jatyadi Ker taila.

Hence a comparative study on Jatyadi ghrita as per classical text and Jatyadi Ker Taila (modified method by using coconut oil replacing ghrita) to know their physicochemical properties was performed. The physicochemical tests performed on different samples of Jatyadi Ghrita and Jatyadi Ker Taila such as specific gravity, acid value, saponification value, Total fatty matter, melting point, viscosity etc. Further standardization by instrument analysis performed on HPTLC for finger printing profile.

MATERIAL AND METHODS

Pharmaceutical study

All the raw materials were procured from the pharmacy and authenticated by Drug testing laboratory in Dept. Rasa Shastra and Bhaishajya Kalpana, National Institute of Ayurveda, Jaipur. Ingredients used in preparation of Jatyadi ghrita and Jatyadi Ker taila are tabulated below.

S. N.	Name of the drugs	Part of use	Quantity	
1.	Murchhita Goghrita (processed ghrita)	Cow's ghee	768 g	1 part-Sneha
1.	Murchhita (processed) Narikel Taila	Coconut oil	768 g	1 part- Sneha
2.	Jatipatra (<i>Jasminum grandiflorum</i>)	Fresh Leaf	35 g	¼ part Kalka Dravya
3.	Nimba patra (<i>Azadirachta indica</i>)	Fresh Leaf	35 g	
4.	Patol patra (<i>Trichosanthes dioica</i>)	Dried Leaf	17.45 g	
5.	Katuka (<i>Picrorhiza kurroa</i>)	Rhizome /Root	17.45 g	
6.	Daruharidra (<i>Berberis aristata</i>)	Stem	17.45 g	Kalka (paste)
7.	Haridra (<i>Curcuma longa</i>)	Rhizome	17.45 g	
8.	Sariva (<i>Hemidesmus indicus</i>)	Root	17.45 g	
9.	Manjishtha (<i>Rubia cordifolia</i>)	Root	17.45 g	
10.	Abhaya (Ushira) <i>Vetiveria zizanioides</i>	Root	17.45 g	
11.	Madhuyashti (<i>Glycyrrhiza glabra</i>)	Root	17.45 g	
12.	Karanja beeja (<i>Pongamia pinnata</i>)	Seeds	17.45g	
13.	Jala	Potable water	3.07 l kg	4 part-Drava
14.	Siktha	Bee's wax	17.45 g	Prakshepa Dravya
15.	Shuddha Tuttha (Copper sulphate)	CuSO ₄	17.45 g	Prakshepa Dravya

Methods

Jatyadi Ghrita was prepared with proper method and standards as per the reference of Ayurvedic Formulary of India (AFI)¹² and Ayurvedic Pharmacopoeia of India (API)¹³ in 3 batches (Jatyadi ghrita -1, Jatyadi ghrita - 2 and Jatyadi ghrita - 3) in the laboratory of Department of Rasa Shastra and Bhaishajya Kalpana, National institute of Ayurveda, Jaipur and same SOP was adopted for preparation of Jatyadi Ker Taila. Jatyadi Ker Taila was also prepared in 3 batches (Jatyadi Ker Taila - 1, Jatyadi Ker Taila - 2 and Jatyadi Ker taila - 3). Because there is no reference found in classics for Murchhana (procedure for removal of Āam and Gandha dosha of tail/ghrita) for Narikel taila (coconut oil) and Jatyadi Ker taila formulation as well; so, all process of Jatyadi Ker Taila preparation had done just like Jatyadi ghrita (only cow ghee was replaced by coconut oil). For preparation of both formulation Kalka (paste) was prepared by 2-12 no. drugs; weight of fresh leaves (drug no 2, 3) was taken double from other dry Kalka dravya.¹⁴ After cleaning them; they made into powder form separately and pass through 250 µm I.S. sieve then added water till the mixture become paste form and grinded to prepare homogeneous blend. Preparation of Jatyadi ghrita was done by Murchhita Goghrita¹⁵, Kalka and Jala on mild heat {between 50⁰ and 90⁰} till Sneha Siddhi Lakshana appeared with intermittent stirring on 1st and 2nd day and continuous stirring on 3rd day. It was filtered through muslin cloth and Prakshepa dravya were added to it. Shodhita Tuttha¹⁶ in 10 ml water were mixed together and passed through muslin cloth. Bees wax was heated with some quantity of ghee on water bath at 100⁰C. The copper sulphate solution was mixed it in melted condition with propeller mixer at 100RPM for 15 min. and remaining Ghrita was added slowly with continuous stirring. Above same procedure was conducted in 3 samples of Jatyadi ghrita and 3 samples of Jatyadi Ker taila (using Murchhita coconut oil).

Analytical study

The physicochemical analysis of Jatyadi ghrita and Jatyadi Ker Taila was conducted on the basis of parameters laid down by CCRAS¹⁷ at Ayush Raj Enterprises Pvt. Ltd. Dehami Begas Road, Mansinghpura Jaipur and S.R. LABS Haldighatimarg, 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 API.¹⁸

HPTLC Fingerprint profile

HPTLC performed by TLC apparatus CAMAGE, Switzerland at Ayush Raj 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.¹⁹

Conditions

Stationary phase: Pre-coated silica gel 60 F₂₅₄ aluminium plates

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

Chamber Saturation Time: 20 min.

Test Solution: 2g of samples of Jatyadi ghrita – 1 and Jatyadi Ker taila -1 were separately extracted by cold maceration in methanol and then filter the liquid extract. Make 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 Linomat 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 254 nm, 366 nm and white light. Process was carried on derivatization of plate by dipping method in anisaldehyde reagent. Dried over 100⁰C for 15 min and visualize in day light. The scanning programme for completely dried plate was prepared at different wavelength of 550 nm, 625 nm and 360 nm. Comparative evaluation of the scanning parameter (densitogram) is performed by final analysis report.

RESULT

Comparative evaluation of physicochemical parameters as showing in Table 1-3 confirmed that both the formulation are not very much differ in their characterization but Melting point of Jatyadi Ker taila is less than Jatyadi ghrita and viscosity is high because of coconut oil base. Results of Physico-chemical parameters follows the range of standards laid down by API for Jatyadi Ghrita formulation and heavy metals are also in permissible limits that shows safety and quality of the formulation. HPTLC study of methanolic extracts of the formulation Jatyadi Ghrita and Jatyadi Ker Taila by inject the prepared sample solutions and qualitative analysis was carried out by comparison with respective peak areas. Samples were analyzed on different wavelengths 550 nm, 625 nm and 700 nm. 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. That shows 8-9 distinct spots of Jatyadi ghrita sample and 11-12 distinct spots of Jatyadi Ker taila formulation. Figure 6 shows Jatyadi ghrita - 1 have 8-9 peaks areas and Jatyadi Ker taila - 1 have 11-13 peak areas. HPTLC fingerprint comparison of Jatyadi ghrita - 1 and Jatyadi Ker Taila - 1 was clearly designated the more spots in Jatyadi Ker Taila at lower R_f values which confirm extraction and stability of more secondary metabolites instead of Jatyadi ghrita formulation. The R_f values of spots differentiate the samples chemical composition and formulation. Also, the visible pattern and colors of spots at 550 nm confirms the samples standardization in visible spectrum. Hence HPTLC Fingerprint profiles confirm standard scanning of samples to generate the standardization parameter for quality control purpose.

Table 1: Organoleptic characteristics of Jatyadi Ghrita (Jatyadi Ghrita) and Jatyadi Ker Taila (Jatyadi Ker Taila) samples

Sample	Color	Odor	Appearance	Clarity
Murchita Ghrita	Yellowish green	Characteristic	Granular, oily	Opaque
Murchhita coconut oil	Greenish yellow	Characteristic	Homogenous	Opaque
Jatyadi Ghrita 1	Greenish yellow	Characteristic	Granular, oily	Opaque
Jatyadi Ghrita 2	Greenish yellow	Characteristic	Granular, oily	Opaque
Jatyadi Ghrita 3	Greenish yellow	Characteristic	Granular, oily	Opaque
Jatyadi Ker Taila 1	Greenish yellow	Characteristic	Homogenous	Opaque
Jatyadi Ker Taila 2	Greenish yellow	Characteristic	Homogenous	Opaque
Jatyadi Ker Taila 3	Greenish yellow	Characteristic	Homogenous	Opaque

Table 2: Comparative Physiochemical analysis of Jatyadighrita (Jatyadi Ghrita) and Jatyadi Ker Taila (Jatyadi Ker Taila)

Physio-chemical Parameters	Different Jatyadi Formulation					
	Jatyadi Ghrita 1	Jatyadi Ghrita 2	Jatyadi Ghrita 3	Jatyadi Ker Taila 1	Jatyadi Ker Taila 2	Jatyadi Ker Taila 3
Acid Value	2.98	2.28	1.91	2.88	3.05	3.21
Rancidity	Slightly rancid	Slightly rancid	Slightly rancid	Slightly rancid	Slightly rancid	Slightly rancid
Unsaponifiable Matter % (w/w)	0.97	0.88	1.02	1.72	1.57	1.16
Saponification value	174.35	151.79	161.82	246.74	246.92	251.01
Total Fatty Matter % (w/w)	88	81.3	84	83	85	80
Ester Value	171.37	149.51	159.91	243.83	243.86	247.8
Refractive Index	1.428	1.428	1.428	1.425	1.426	1.426
Specific Gravity	0.925	0.930	0.916	0.927	0.924	0.928
Viscosity	47cp (At 40°C)	-	-	76cp (At 20°C)	-	-
Iodine value	32.47	25.94	20.29	36.37	28.90	34.19
Peroxide value	5.08	7.48	4.75	6.75	6.22	3.76
Melting Point	39°C	-	-	27°C	-	-
Heavy Metal Analysis						
Lead	ND	-	-	ND	-	-
Cadmium	0.038 ppm	-	-	0.0391 ppm	-	-
Mercury	ND	-	-	ND	-	-
Arsenic	ND	-	-	ND	-	-

Table 3: R_f values obtained from HPTLC fingerprint profile of Jatyadi Ghrita and Jatyadi Ker Taila

Scanning Wavelengths	Rf Values of Major Spots	
	Jatyadi Ghrita - 1	Jatyadi Ker Taila - 1
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

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

Figure 1: HPTLC banding pattern at 254 nm of Jatyadi Ghrita (JG) - 1 (First 3 track from Left to right) and Jatyadi Ker Taila (JKT) - 1 (Last 3 tracks)

Figure 2: HPTLC banding pattern at 366 nm of Jatyadi Ghrita (JG) - 1 (First 3 track from Left to right) and Jatyadi Ker Taila (JKT) - 1 (Last 3 tracks)

Figure 3: HPTLC banding pattern at 366 nm of derivatized plate for Jatyadi Ghrita (JG) - 1 (First 3 track from Left to right) and Jatyadi Ker Taila (JKT) - 1 (Last 3 tracks)

Figure 4: HPTLC banding pattern at white light of Jatyadi Ghrita (JG) - 1 (First 3 track from Left to right) and Jatyadi Ker Taila (JKT) - 1 (Last 3 tracks)

Figure 5: HPTLC banding pattern at white light of derivatized plate for Jatyadi Ghrita (JG) - 1 (First 3 track from Left to right) and Jatyadi Ker Taila (JKT) - 1 (Last 3 tracks)

Figure 6: Scanning pattern of the entire track showing significant difference between Jatyadi Ghrita - 1 and Jatyadi Ker Taila - 1 formulation

CONCLUSION

By the above study it can conclude that; coconut oil (Ker taila) can be used better substitute of Ghrita as an oleaginous base for the preparation of Jatyadi formulation. Related physico-chemical tests and HPTLC profile clearly shows the more stability of formulation and increased number of secondary metabolite extraction in coconut oil than ghee. Possibly the above results denote better substitution of classical formulation Jatyadi Ghrita is Jatyadi Ker Taila (by using coconut oil replacing Ghrita). Further clinical study should be carried out in support of above Pharmaceutico-analytical study.

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