

**PHARMACEUTICO-ANALYTICAL STUDY OF DHĀNVAÑTARA
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Corresponding Author*Dr. L. K. Virajitha**PG Scholar, PG Department
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Venkateswara Ayurvedic
College, Tirupati.**ABSTRACT**

Analytical study of Ayurvedic preparations is the need of present scientific era. Though the Ayurvedic drugs are time tested and have been used successfully in the management of various ailments, it is now necessary to prove their quality, efficacy and safety to the scientific world through various modern analytical parameters. Dhānvañtara ghṛta, mentioned in Aṣṭāṅga Hṛdaya Pramēha Cikitsa is a poly herbal formulation containing 37 ingredients and is indicated for Snēhapāna in Madhumēha. Madhumēha (Diabetes Mellitus) is a disease in which the urine excreted possesses the quality similar to that of Madhu (honey) in its colour, taste, smell and consistency. In the

pathogenesis of Madhumēha, Bahu drava śleṣma (Kapha with excess fluid component) and Bahu abaddha dūṣyās (excess abaddha mēda, māmsa, vasā, majja, klēda, śukra, rakta, lasika, rasa and ojas) play an important role. So, in its management such drugs have to be selected which are against Kapha, Meda and Kleda. Dhānvañtara Ghṛta is one such formulation, which has Kaṭu, Tikta, Kaśāya Rasa; Laghu, Rūkṣa Guṇa producing Rūkṣaṇa effect as it has opposite qualities to that of Kapha and Medas, which are the main entities in the pathogenesis of Madhumēha. In the present study, an attempt has been made to prepare Dhānvañtara ghṛta and standardise it through analytical parameters like organoleptic properties, Moisture content, Specific gravity, Refractive index, Rancidity test, Acid value, Saponification value, Iodine value, Peroxide value, Richert Meissl value, Polenske value, Viscosity and High performance thin layer chromatography (HPTLC). All the parameters were found to be good and within the standards.

KEYWORDS: Analytical standardization, Dhānvañtara ghr̥ta, Pramēha, Diabetes Mellitus, HPTLC.

INTRODUCTION

Diabetes Mellitus, a silent epidemic and a potentially life threatening life style disorder, is considered as one of the arch enemy of the mankind and has always been invincible. In the present era, over stress, sedentary life style, rapid urbanization, unhealthy diet and obesity has led to an upsurge in many life style disorders and Diabetes is one among them. In Ayurveda, Pramēha which can be correlated with Diabetes is characterized by Prabhūta mūtrata (increased quantity of urine) and Avila mūtrata (turbidity of urine).^[1] Ācārya Caraka classified Pramēha based on the Doṣa involved into 20 types - Kaphaja Pramēha, which is of 10 types, Pittaja Pramēha, 6 types and Vātajaja Pramēha, 4 types. Madhumēha is one among the Vātajaja Pramēhas and is characterized by similar to that of Madhu (honey) in its colour, taste, smell and consistency.^[2] Ācārya Suśr̥ta opines that all the varieties of Pramēha when left untreated, will change into Madhumēha.^[3] In the pathogenesis of Madhumēha, Bahu drava śleṣma (Kapha with excess fluid component) and Bahu abaddha dūṣyās (excess abaddha mēda, māmsa, vasā, majja, klēda, śukra, rakta, lasika, rasa and ojas) play an important role.^[4] So, in its management such drugs have to be selected which are against Kapha, Meda and Kleda. Dhānvañtara Ghr̥ta^[5] is one such formulation, which has Kaṭu, Tikta, Kaśāya Rasa; Laghu, Rūkṣa Guṇa producing Rūkṣaṇa effect as it has opposite qualities to that of Kapha and Medas, which are the main entities in the pathogenesis of Madhumēha. Standardization of Ayurvedic formulations is an important step for establishment of biological activity, consistent chemical profile, or quality assurance for production. In the present study, Dhānvañtara ghr̥ta was prepared by referring the method described in the text Aṣṭāṅga Hṛdaya Pramēha Cikitsa and further studied organoleptically, physico-chemically and chromatographically for developing standards.

MATERIALS AND METHODS

The process was carried out in two steps

1. Pharmaceutical Study
2. Analytical Study

1. Pharmaceutical study

Dhānvañtara ghr̥ta for the present clinical study was prepared in Vaidya Ratnam Ayurveda Research Institute, Vaidya Ratnam Oushadashala Pvt, Ltd, Ollur, Thaikkattusery, Thrissur,

Kerala. All the ingredients were purchased from Kerala local market. Foreign matter adhering to raw drugs was removed and cleaned and Shodhana of raw materials was done where necessary. The base which was used for the preparation of this Ghrta, i.e., Go ghrta was also purchased from the local market.

Table no. 1: Ingredients and Quantity of dhānvañtara ghr̥ta.

Kaśāya dravya - each 8.75 kg	
Kāśmarī - <i>Gmelina arborea</i>	Bilva - <i>Aegle marmelos</i> Linn.
Pāṭalā - <i>Stereospermum suaveolens</i>	Śyonāka - <i>Oroxylum indicum</i>
Agnimanthā - <i>Clerodendrum phlomidis</i>	Śālaparṇi - <i>Desmodium gangeticum</i>
Prśniparṇi - <i>Uraria picta</i>	Brhatī - <i>Solanum indicum</i>
Nidigdhikā - <i>Solanum surattense</i>	Gokṣura - <i>Tribulus terrestris</i>
Śaṭhī - <i>Hedychium spicatum</i>	Dantī - <i>Baliospermum montanum</i>
Surāhva - <i>Cedrus deodara</i>	Punarnavā - <i>Boerhaavia diffusa</i>
Snuhī - <i>Euphorbia neriifolia</i>	Arkamūla - <i>Calotropis procera</i>
Pathyā - <i>Terminalia chebula</i>	Bhukadamba - <i>Sphaeranthus indicus</i>
Āruṣkara - <i>Semecarpus anacardium</i>	Karañjamūla - <i>Pongamia pinnata</i>
Varuṇamūla - <i>Crataeva nurvala</i>	Pippalīmūla and pippalī - <i>Piper longum</i>
Puṣkaramūla - <i>Inula racemosa</i>	Yava - <i>Hordeum vulgare</i>
Kola - <i>Zizyphus jujube</i>	Kulattha - <i>Dolichos biflorus</i>
Kalka dravya - 0.2625 kg each	
Cavya - <i>Piper chaba</i>	Vacā - <i>Acorus calamus</i>
Nicula - <i>Barringtonia acutangula</i>	Rohiṣa - <i>Cymbopogon martini</i>
Tṛvṛt - <i>Operculina turpethum</i>	Viḍaṅga - <i>Embelia ribes</i>
Kampillaka - <i>Mallotus philippinensis</i>	Bhāraṅgī - <i>Clerodendrum serratum</i>
Viśvā - <i>Zingiber officinale</i>	
Go Ghrta- 35 kg	
Final Output- 31.5 kg	

Procedure

- Ingredients of Kalka in said ratio were weighed, powdered and kept separately.
- Ingredients of Kaśāya in said ratio (1 part) was weighed and Kaśāya was prepared by boiling in 16 parts of water and reduced to 4 parts.
- Kalka and the Drava dravya were mixed together, then Sneha was added, boiled and stirred continuously so that the Kalka is not allowed to adhere the vessel. Care was taken to determine the proper stages of Sneha pāka.
- When all the Drava dravyas had evaporated, at this stage, it was stirred more often and carefully to ensure that the Kalka does not stick to the bottom of the vessel. The Kalka was taken out from the ladle and tested from time to time to know the condition and stage of the pāka.

- After Sneha pāka in Madyama paka (Formation of varthi with minimal moisture percentage in kalka) process, in order to obtain optimum quantity of Sneha, the Kalka was squeezed at hot stage only.
- Once the Ghrta had self-cooled, it was filtered and bottled.



Fig. 1.1: Kashaya preparation.



Fig.1.2: Boiling kashaya, Kalka, Ghrta.



Fig. 1.3: Reducing the mixture by boiling.



Fig. 1.4: Separation of Kalka.



Fig. 1.5: Separation of ghrta.



Fig. 1.6: Paka pariksha.



Fig. 1.7: Paka siddhi lakshana.



Fig. 1.8: Filtering of ghrta.

Fig. 1: Steps in the preparation of dhānvañtara ghr̥ta.

2. Analytical study

Organoleptic tests

Organoleptic studies refer to evaluation of a drug by means of different organs of the human body or by sense, such as appearance of the drug material, its odour and taste. The organoleptic characteristics of the prepared Dhānvañtara Ghr̥ta were as follows –

Table no. 2: Organoleptic characteristics of dhānvañtara ghr̥ta.

Parameter	Observation
Nature	Viscous
Colour	Brown
Smell	Characteristic
Taste	Characteristic

Physico-Chemical tests

Physico-Chemical tests deal with primary physical and chemical properties of a sample, which can hint about the internal molecular behaviors at different natural conditions. It helps in understanding the stability of a drug when it is stored for long time. The analysis of Physico – chemical parameters of Dhānvañtara Ghr̥ta was done according to the methods described in Ayurvedic Pharmacopoeia of India (API).

- a. **Moisture content**^[6]: It is the loss in weight of sample after heating at 105°C until it attains constant weight. Moisture content determines the quantity of moisture a sample contains. The stability, shelf life and microbial safety depend on this value.
- b. **Specific gravity**^[7]: The specific gravity of a liquid is the weight of a given volume of the liquid at 25° (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air.

- c. **Refractive index**^[8]: The refractive index of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.
- d. **Rancidity test**^[9]: Rancidity is a condition produced by aerial oxidation of unsaturated fat present in foods and other products, marked by unpleasant odour or flavour.
- e. **Acid value**^[10]: The acid value is the number of mg of *potassium hydroxide* required to neutralize the free acids in 1 g of the substance.
- f. **Saponification value**^[11]: The saponification value is the number of mg of *potassium hydroxide* required to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat.
- g. **Iodine value**^[12]: The Iodine value of a substance is the weight of *iodine* absorbed by 100 part by weight of the substance.
- h. **Peroxide value**^[13]: The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.
- i. **Richert meissl value**^[14]: The Reichert-Meissl value is the number of millilitres of 0.1N aqueous sodium hydroxide solution required to neutralize steam volatile water soluble fatty acids distilled from 5g of an oil/fat under the prescribed conditions. It is a measure of water soluble steam volatile fatty acids chiefly butric and caprole acids present in oil or fat.
- j. **Polenski value**^[14]: The Polenske value is the number of millilitres of 0.1N aqueous alkali solution required to neutralize steam volatile water insoluble fatty acids distilled from 5 g of the oil/fat under the prescribed conditions. It is a measure of the steam volatile and water insoluble fatty acids, chiefly caprylic, capric and lauric acids present in oil and fat.
- k. **Viscosity**: Viscosity is the measure of resistance to graduated deformation by shear stress or tensile stress.

3. High performance thin layer chromatography (HPTLC)

TLC plates consisted of 5.0 × 10.0 cm; precoated with silica gel 60 F 254 TLC plates (E. Merck KGaA) were used. The spotting was carried out with the help of CAMAG Linomat 5 “Linomat5_171118”. Automatic Sample Spotter (Camag Muttenez, Switzerland); mounted with the 100 µL syringe. Application parameters were the length of the band was 8 mm, distance between bands was 12.5 mm, the application position along Y axis was 10 mm, and the start position along X axis was 12.5 mm, application rate of 150 nL/s. Linear ascending

development was carried out in CAMAG glass twin trough chamber (20 × 10 cm) covered with stainless steel lid. The densitometer consisted of a CAMAG TLC Scanner linked to winCATS Software. The slit dimensions were 10.00 × 0.60 mm (Macro) and the scanning speed 20 mm/s. The optimized chamber saturation time for mobile phase was 30 min and the plates were developed up to 80 mm using the solvent systems Toluene : Ethyl Acetate : Hexane 60:30:10 (v/v) as a mobile phase. The average development time was 20 min. After development the plate was dried for 2 min with Hair Dryer at 60° C temperature and optical densitometric scanning at $\lambda_{\text{max}} = 366 \text{ nm}$ was performed. After densitometric scanning, chromatograms were evaluated via peak area. Scanned peak areas were recorded for each sample at each concentration level.

Simultaneous quantification of markers

For the quantification 10 μL of sample solutions were spotted on a TLC plate. The plates were developed and scanned. The peak areas were recorded and the amount of all markers was calculated using the calibration curve.

Sample preparation for dhānvañtara ghṛta: Sample Preparation for Dhānvañtara Ghṛta was optimized to extract the marker compounds efficiently and also to achieve good fingerprinting. The sample solutions were prepared as given below:

Accurately weighed 0.2 μL of Dhānvañtara Ghṛta was extracted with 10 mL of methanol and 20 mL of hexane by means of separating funnel. The mixture was shaken vigorously and kept it for 5 min for separating the two layers. To get the sample free from fat, methanolic layer was treated with 10 mL hexane. Hexane layers were leftover. The volume was made up to 25 mL with methanol by using volumetric flask and filtered through 0.45 μm membrane syringe filter. This solution was applied on TLC plate for HPTLC analysis.

RESULTS

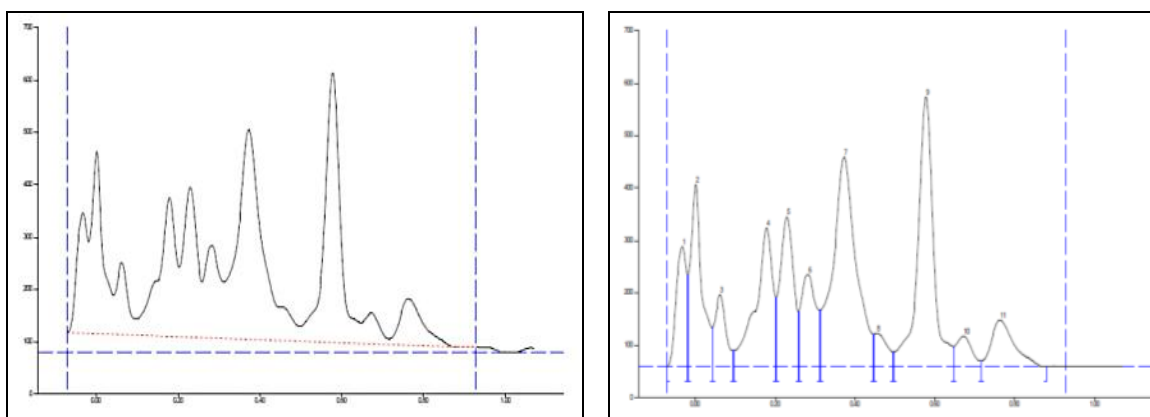
Table no. 3: Results of Physico – chemical tests.

S. no.	Parameter tested	Result
1.	Moisture content	0.18%
2.	Specific gravity	0.9144
3.	Refractive index	1.455
4.	Rancidity test	Negative
5.	Acid value	1.74
6.	Saponification value	224
7.	Iodine value	33.88
8.	Peroxide value	3.32

9.	Richert meissl value	27.31
10.	Polenske value	0.3
11.	Viscosity	9278

Interpretation of HPTLC Report of dhanvantara ghrta

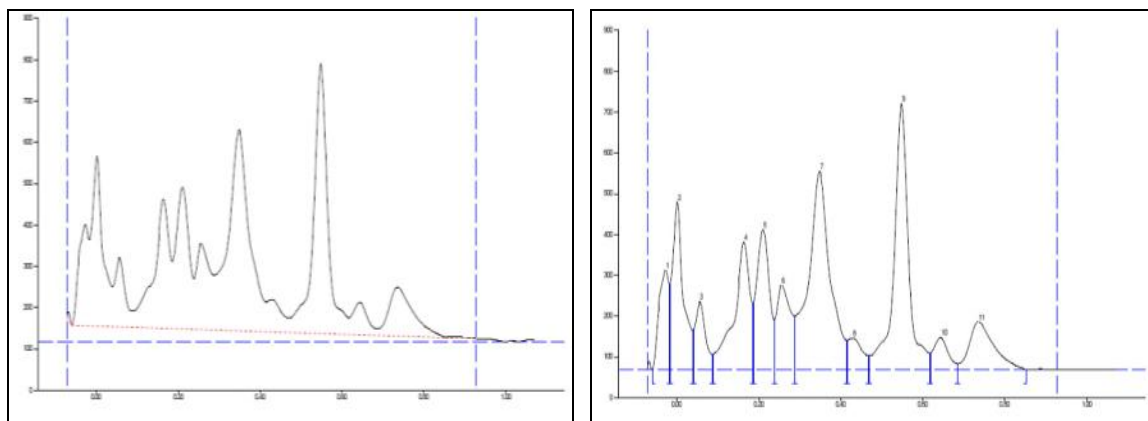
- At short UV 254nm, 11 bands are observed at Rf values of -0.03, 0.04, 0.06, 0.18, 0.23, 0.28, 0.37, 0.46, 0.58, 0.67 and 0.77 with Dark colour intensity.
- At long UV 366nm, 11 bands are observed at Rf values of -0.03, 0.02, 0.06, 0.17, 0.21, 0.26, 0.35, 0.43, 0.55, 0.65 and 0.74 with colour intensities of dark blue.
- Consecutive maximum areas covered are 20.07%, 21.08%, 20.21% with the Rf values of 0.58, 0.55, 0.54 respectively.



Graph no. 1.

Track 1: Dhanvantara Ghrta.

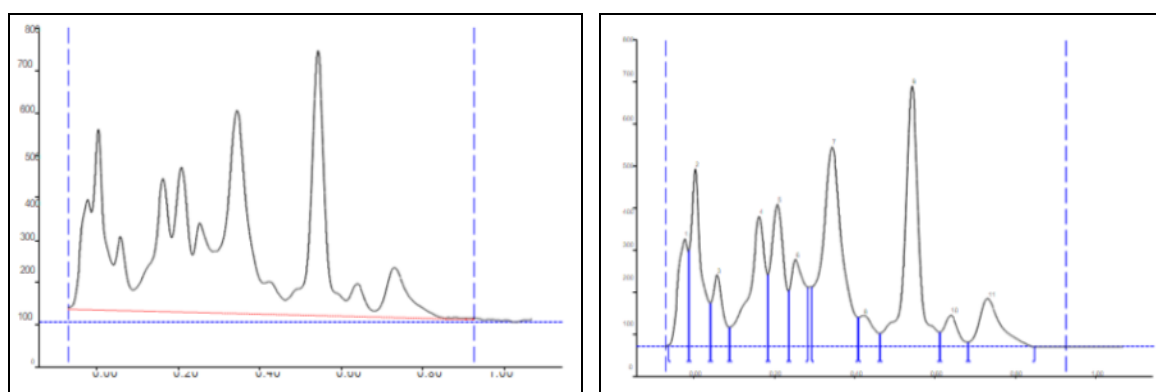
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	0.3	-0.03	229.5	8.94	-0.02	175.2	4870.9	5.97	unknown *
2	-0.02	175.3	0.00	347.5	13.54	0.04	74.4	7960.2	9.75	unknown *
3	0.04	75.6	0.06	137.5	5.36	0.10	30.6	3144.8	3.85	unknown *
4	0.10	30.6	0.18	264.8	10.32	0.20	132.3	9296.3	11.39	unknown *
5	0.20	132.4	0.23	285.7	11.13	0.26	105.2	7948.2	9.73	unknown *
6	0.26	106.5	0.28	175.6	6.84	0.31	108.0	5230.6	6.41	unknown *
7	0.31	108.2	0.37	399.7	15.57	0.45	62.8	18967.9	23.23	unknown *
8	0.45	62.9	0.46	64.2	2.50	0.50	28.6	1740.7	2.13	unknown *
9	0.50	28.6	0.58	515.2	20.07	0.65	38.9	16486.8	20.19	unknown *
10	0.65	39.0	0.67	58.6	2.28	0.72	11.0	1841.6	2.26	unknown *
11	0.72	11.1	0.77	89.0	3.47	0.88	0.0	4165.5	5.10	unknown *



Graph no. 2.

Track 2: Dhanvanthara ghrita.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.06	2.5	-0.03	245.2	7.92	-0.02	208.2	4756.2	5.16	unknown *
2	-0.02	208.4	0.00	410.7	13.26	0.04	97.1	8930.7	9.69	unknown *
3	0.04	98.4	0.06	167.3	5.40	0.09	38.2	3491.6	3.79	unknown *
4	0.09	38.4	0.17	311.8	10.07	0.19	162.0	10016.1	10.87	unknown *
5	0.19	164.8	0.21	342.4	11.06	0.24	122.2	8659.9	9.40	unknown *
6	0.24	123.9	0.26	208.1	6.72	0.29	132.1	5828.5	6.33	unknown *
7	0.29	132.2	0.35	484.7	15.65	0.42	72.1	20928.3	22.71	unknown *
8	0.42	72.2	0.43	77.7	2.51	0.47	34.1	2263.3	2.46	unknown *
9	0.47	34.1	0.55	652.8	21.08	0.62	40.3	19164.7	20.80	unknown *
10	0.62	40.7	0.65	79.0	2.55	0.69	15.0	2331.5	2.53	unknown *
11	0.69	15.0	0.74	116.9	3.77	0.85	0.1	5764.7	6.26	unknown *



Graph no. 3.

Track 3: Dhanvanthara ghrita.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	EndRf	End Height	Area	Area %	Assigned substance
1	-0.06	4.5	-0.02	258.4	8.38	-0.01	227.7	5320.8	5.91	unknown *
2	-0.01	231.8	0.00	424.7	13.78	0.04	104.5	8735.8	9.70	unknown *
3	0.04	104.9	0.06	172.5	5.60	0.09	47.5	3771.4	4.19	unknown *
4	0.09	47.5	0.16	311.9	10.11	0.18	172.5	10249.8	11.38	unknown *

5	0.19	173.9	0.21	340.0	11.03	0.24	133.5	8738.9	9.70	unknown *
6	0.24	135.6	0.25	209.8	6.80	0.28	142.5	5889.4	6.54	unknown *
7	0.29	143.4	0.34	476.8	15.46	0.41	70.9	19757.8	21.94	unknown *
8	0.41	71.2	0.42	74.9	2.43	0.46	32.7	2191.9	2.43	unknown *
9	0.47	33.0	0.54	623.1	20.21	0.61	34.8	18041.1	20.03	unknown *
10	0.61	34.9	0.64	75.3	2.44	0.68	11.9	2213.8	2.46	unknown *
11	0.69	12.0	0.73	115.9	3.76	0.85	0.3	5155.0	5.72	unknown *

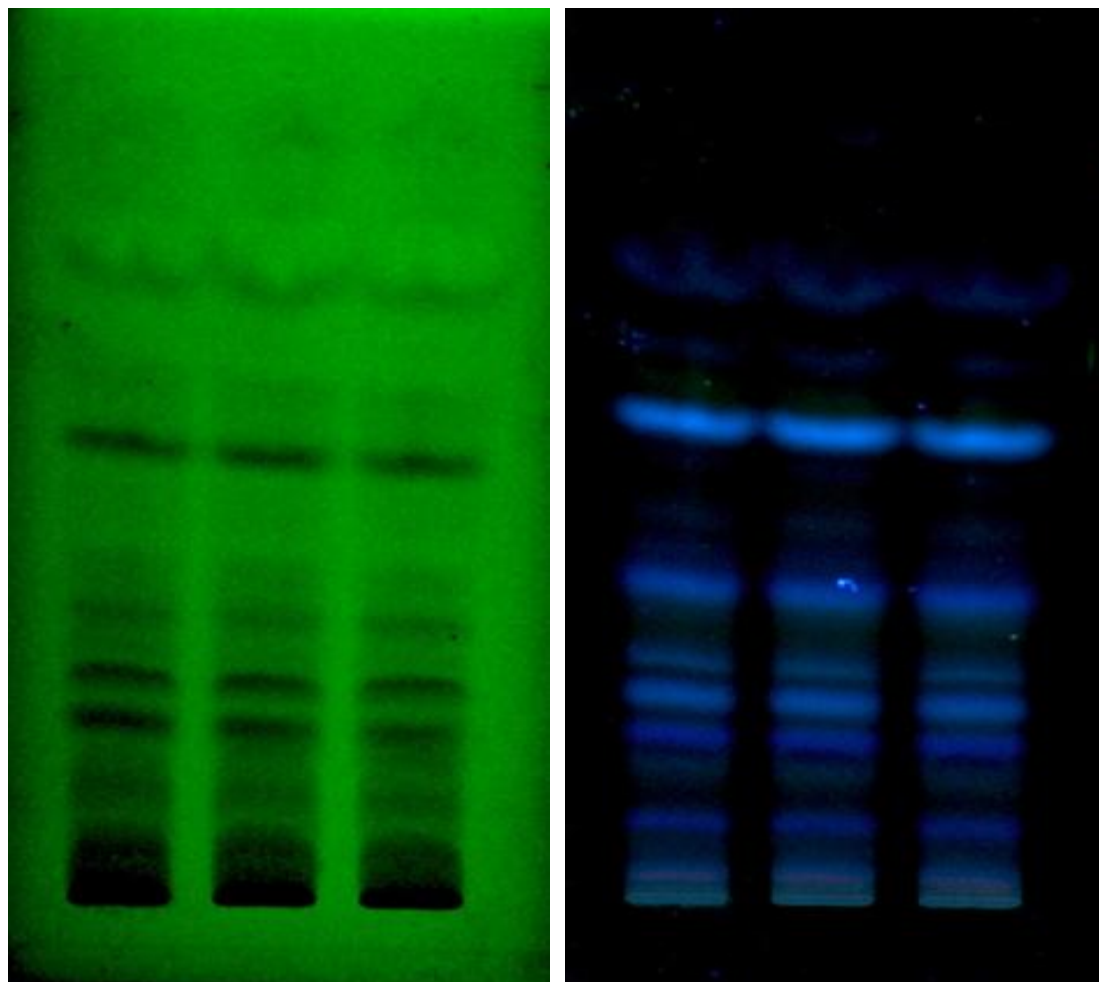


Fig. 2: Showing result of HPTLC of Dhanvantara Ghrta at wavelengths 254 and 366 nm.

DISCUSSION

1. Pharmaceutical study

During the preparation of ghr̥ta, the siddhi lakṣaṇās observed were

- Varti formation of Kalka
- Disappearance of foam
- Specific dark yellowish-brown colour of Ghr̥ta observed.
- Characteristic odour of Ghr̥ta observed.

Precautions taken during the preparation of ghr̥ta are

- Vessel used was clean, heavy bottom and of adequate size & volume.
- Mandāgni was maintained throughout the process.
- Continuous stirring was employed to avoid carburizing of kalka & facilitate evaporation.
- Timely performance of Pāka Lakṣaṇa and observations of Siddhi Lakṣaṇa were done.
- After the process, Ghṛta was filtered in warm condition to avoid the loss.
- Clean cloth was used for filtering.
- The consistency and colour were noted from time to time.

2. Analytical study

Analytical study is an essential part of any research work. It provides us with experimental data (qualitative and quantitative) and makes us know about certainty of our assumptions and prevents from wrong interpretations. It provides us with the complete knowledge of our formulation like identity, size, structure of chemical constituents and physical properties. It hints us about toxic properties of drugs, if any.

Organoleptic tests

- The organoleptic characteristics of the prepared Dhānvañtara Ghṛta were viscous in nature, brown coloured liquid, with characteristic smell and taste.
- Organoleptic tests helps in providing basic information about the drug that can be done by one's sensory organs.
- It plays a major role when the medicine is ingested orally.
- Brown colour of the final product is due to the boiling of Kaṣāya and Kalka dravyas in Ghṛta.
- Unctuous on touch of the formulation indicates the proper preparation of Ghṛta.

Physico- Chemical tests

Investigating Physico-Chemical systems makes possible to determine the nature of interactions between the components of sample through a study of relationship between sample physical properties and composition.

a. Moisture content

Moisture content signifies the amount of residual water in the finished product. Temperature was set to 105°C to facilitate complete evaporation of water. This value determines the quantity of moisture a given sample contains. Stability, shelf life and microbiological safety

depend on this value. The sample of Dhānvañtara ghṛta was found to have moisture content of 0.18%.

b. Specific gravity

The presence of dissolved substances in Snēha is expected to change its specific gravity. So it is considered to be an important parameter for analyzing medicated Snēha. This helps us to access the molecular information in a non-invasive way. The specific gravity of Dhānvañtara Ghṛta was 0.9144.

c. Refractive index

Refractive index is a fundamental physical property of a substance often used to identify a particular substance, confirm its purity, or measure its concentration. Refractive index of prepared Dhānvañtara Ghṛta is 1.455.

d. Rancidity test

Rancidity is a condition produced by aerial oxidation of unsaturated fat present in foods and other products, marked by unpleasant odour or flavour. The free fatty acids in a fat compound are responsible for the Rancidity of the compound. The Rancidity test of prepared Dhānvañtara Ghṛta was negative.

e. Acid value

The acid value indicates presence of free fatty acid in the oil which is responsible of rancidity of compounds; higher the free fatty acid more is the rancidity, this helps to decide the shelf life of the sneha. The Acid value of prepared Dhānvañtara Ghṛta was found to be 1.74.

f. Saponification value

Saponification value is a measure of the average molecular weight of all the fatty acid present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. Saponification value of Dhānvañtara Ghṛta was 224.

g. Iodine value

The Iodine value is a measure of degree of unsaturation of fat. The more the Iodine number, more the number of unsaturated fatty acid bonds present. When more Iodine is attached, the compound is more reactive, less stable and more susceptible to oxidation. The susceptibility to rancidity increases with Iodine value. The Iodine value of prepared Dhānvañtara Ghṛta was 33.88, indicating less chances of Rancidity and otherwise also indicating more stability of Dhānvañtara Ghṛta.

h. Peroxide value

Peroxide value signifies the percentage of oxidation of the Ghṛta and Taila. It helps us to find the stability of the sample. If the peroxide value is more, it shows more oxidation and chances of attaining rancidity is also more. Peroxide value of Dhānvañtara Ghṛta was found to be 3.32, which indicates that Dhānvañtara Ghṛta has more stability.

i. Richert meissl value

Higher content of volatile fatty acids of a compound is responsible for its higher Richert Meissl number. It is useful in testing the purity / adulteration of fat. The Richert Meissl value of prepared Dhānvañtara Ghṛta was found to be 27.31.

j. Polenske value

Polenske value is a measure of the steam volatile and water insoluble fatty acids, chiefly caprylic, capric and lauric acids present in oil and fat. It is an indicator of how much volatile fatty acid can be extracted from fat through saponification. The Polenski value of prepared Dhānvañtara Ghṛta was 0.3.

k. Viscosity

Viscosity is the measure of resistance to graduated deformation by shear stress or tensile stress. The viscosity of the prepared Dhānvañtara Ghṛta by Brookfield method was 9278.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC helps in qualitative and quantitative analysis of herbal drugs. Standardization is an important step for the establishment of a consistent chemical profile of an herbal drug. HPTLC is an important tool in the standardization of herbal medical product.

CONCLUSION

Dhānvañtara Ghṛta is one of the Ghṛta preparations mentioned for treatment of Madhumēha. The detailed method of preparation is available in Aṣṭāṅga Hṛdaya Pramēha Cikitsa. With that classical reference in backdrop, Dhānvañtara Ghṛta was prepared in lines with standard operating procedures (SOP) and subjected for different analysis thereon. The result of analytical study with HPTLC has been proposed as a monograph to identify and check the quality of Dhānvañtara Ghṛta.

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