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Standardization of Hinguvadhi Ghrita W.S.R Pharamacmaceutico Analytical Study

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ABSTRACT
Background: In the present era, there is a need for examine the process of systematic research methodology and to provide genuine finding of various ayurvedic formulation mentioned in ayurvedic classics for the acceptance.
Objective: To Standardize Hinguvadhi Ghrita on various parameter to assure its safety and efficacy w.s.rpharmaceutico analytical Study.
Method: Hinguvadhi Ghrita was analyzed for its organoleptic physico-chemical features and screened for phyto-constituents as well as its chromatographic analysis.
Result: The standardization and pharamaceutic analytical study of the ghrita helps to lay safety and efficacy of the ayurvedic formulation, hinguvadhi Ghrita.
Conclusion: The analytical methodology in specific basis for research in ayurvedic formulation will facilitate the researchers to set quality standards as well as parameters to fulfill the requirement of therapeutic efficacy, safety and purity of ayurvedic ghee formulation. Hence, in this present study, it has been decided to standardize Hinguvadhi Ghrita.

Keywords: Hinguvadhi Ghrita, Standardization, Pharmaceutics- analytical study, Medicated ghee, Parameter.

INTRODUCTION

The Process of developing and agreeing upon technical standards that provides numerical value which qualifies the parameter, denoting the quality of formulation is known as standardization. In ayurvedic pharmacopoeia and formulatory, various ayurvedic formulations such as vati, churna, lehya, asava, arista, kashaya, ghrita have been explained forvarious disorders. Ghrita are lipid based ayurvedic formulation or medicated ghee in which ghee is boiled with decoction or paste of the crude drug so that the active constitution of drugs gets transferred into the ghrita[1]. As per Ayurveda, ghrita is said to be the healthiest option of edible fat as it is facilitated by the active constituents that added to it in the medicated ghee formulation[2].Drug administered in the form of ghee are quickly digested and absorbed[3]. The ayurvedic formulation mentioned in ayurvedicclassic for various disorder need a scientific validation for adopting research methodology. Standardization of drug is the first step for scientifically based research. The present study was undertaken to ascertain the authenticity of all the ingredients of Hinguvadhighrita and presence of components as recommended through pharmacogenetic study and physicochemical analysis of Hinguvadhighrita[4,5]. As per the method and formula given in the Ayurvedic Formulary, the ghrita was prepared from the purified and standardized crude herbal constituents. In order to assure its safety and efficacy on various grounds, it was standardized. Standardization is beneficial for laying a solid scientific validation with the help of new technology. Hinguvadhighrita is described in samhitas with different name and slight change in content. Chakarpani has mentioned Hinguvadhighrita in Amavata Chikitsa Chapter which is having broad indication such as indigestion, pain, constipation, abdominal distention, rheumatoid Arthritis, low back pain, grahani, appetizer, malabsorption syndrome[6] etc.

Collection of raw Drugs

Raw Drugs were collected from the rural farms of Chennai at the time of preparation of medicine. All the drugs were identified and authenticated from CCRAS, Chennai for the purpose of Pharmacogenetic study. The physical impurities were removed from the herbal drugs and they were dried and made into a course powder. HinguvadhiGhrita was prepared as perclassical reference given by Acharya Charkapani in Amavata Chikitsa Chapter. A Physio chemical analysis of the final product was carried out in Captain Srinivasa Murthy Central Ayurveda research Institute, CCRAS, Arumbakkam, Chennai.

Table 1: Composition of Hinguvadhi Ghrita

S.no	Ingredients	Botanical Name	Parts used	Quantity
1	Hingu	FerulaAsafoetida	Resin	1 part
2	Sunthi	Zingiber Officinale	Dried Rhizome	1 part
3	Pippali	Piper Longum	Fruit	1 part
4	Maricha	Piper Nigrum	Fruit	1 part
5	Chavya	Piper Retrofractum	Root	1 part
6	Saindhava	Rock Salt	Mineral	1 part
7	Go Ghrita	ButyrumDepartum	Whole part	4 part

Fig. 1: Ingredients of Hinguvadhighrita

Method of preparation of Hinguvadhighrita
Reference: Chakradatta, Amavata chikitsa Prakaran
Materials: After collecting all the ingredients of HinguvadhiGhrita as mentioned in **Table 1**, The Ghrita was prepared according to the Sneha Kalpana mentioned in Sarangadhara Samhita[7]
Raw hingu was pounded in an iron mortar and pestle into small pieces of green gram size and fried deeply in an iron fry pan (paalika Yantra) using equal quantity of cow ghee. It was fried till it turns into light weight and dark brown in color.
Shunti, Maricha, Pippali, Hingu and Saindhava was powdered individually and sieved (80 mesh size). Then it is homogenously mixed into a fine bolus(kalka) in the porcelain mortar and pestle. Cow ghee was taken in a thick bottom vessel and it will be heated in low flame. The bolus(kalka) was added to ghee on appearance of slight fumes and then kanji was added and stirred to obtain homogenous form. The mixture was boiled in low to medium flame with intermediate stirring till it combined with the testing criteria with fulfilment of wick and flame test, Hinguvadhighrita was filtered in a muslin cloth and will be stored in an air tight glass jar after cooling.

Fig.2: Preparation of Hinguvadhi ghritam

Fig. 3: Hinguvadhighrita

Organoleptic Study[8,9,10]:
To evaluate the organoleptic characteristic of a drug by color, odor, size, shape, taste and special features including touch, texture, odor etc was carefully noted and illustrated in **Table2**.

Table 2: Showing results of Organoleptic characters of HinguvadhiGhrita

S.no		
1	Colour	Light Green
2	Odour	Ghee like
3	Consistency	Semi liquid
4	Apperance	Oily viscous
5	Taste	Bitter
6	Touch	Sticky

Physio chemical Analysis:

Physio chemical analysis has been done as described for ghrita formulation of the Ayurvedic Pharmacopia of India. Standard procedure will follow during physiochemical Analysis of HinguvadhiGhrita.

Determination of Refractive Index[11]: The ratio of the velocity of light in vacuum or air, to that in the substance is known as Refractive Index. The Refractive index of the Hinguvadhighrita is measured by Abbe's refractometer using a monochromatic light source, the apparatus is calibrated with water as the liquid. Refractive Index of Hinguvadhighrita was determined by 1 drop of sample. Refractive Index value is affected by the temperature and degree of saturation. The data found in samples of Hinguvadhighrita is in limit according to pharmacopeia standards.

Determination of Specific Gravity[12]: The weight of a given volume of the substance at the status temperature as compared with the weight of an equal volume of water at the same temperature, weighing being taken in air is known as specific gravity. The weight of lipid material is affected by the factors such as basic constitutions, dissolved constitution used in the preparation of ghrita. Fat may also change with thermal fluctuation. The result was complying with API specification.

Determination of Rancidity[13]: It is a process of formation of unpleasant odor, taste as a result of moisture oxygen of air and enzyme. The result shows positive rancidity of the preparation.

Determination of Acid Value[14]: It is the number of potassium hydroxide in milligram required to neutralize the free fatty acids present in 1gm of fat. Acid value varies linearly with rancidity. The result of acid value of hinguvadhighrita under the normal limit (not more than3) according to pharmacopoeia standard for Ayurvedic formulation, CCRAS.

Determination of saponification value[15]: saponification =mg of KOH consumed by 1g of fat (The number of mg of potassium hydroxide required to neutralize the free acids and saponify the 1gm of the sample material. The saponify value of Hinguvadhighrita is nearby the normal limit.

Determination of Iodine Value[16]: Iodine value is said to be number of grams of iodine absorbed by 100gm of the sample when determined by using wijas solution. The dissociation and unsaturation of the molecules of compound is increased by heat. The value of hinguvadhighrita was found to be unsaturated.

Determination of Loss of Drying at 110 C[17]: For 2hrs, 5gm weighed crucible material is placed in tilted lid position in the oven at 105^0C. Deterioration time of the plant material depends upon the amount of water present in plant material. The plant can be easily deteriorated due to fungus, if the water content is high.

Determination of PH value[18]: The PH value of an aqueous liquid is defined as the common logarithm of the reciprocal off hydrogen ion concentration expressed in gram per liter. The result is slightly more acidic in nature.

Determination of Peroxide[19]: The reactive oxygen contents expressed in terms of milli equivalents(meq) of free iodine per kilogram of fat is known as the peroxide value (POV). The result shows the standard limit of Pharmacopoeia standard for Ayurvedic Formulation, CCRAS.

Determination of Total Fatty Acid[20]: Normally Fatty Acids are formed during the decomposition of glycerides oil. So, it is relatively measure of rancidity. The content of free fatty acid is expressed by oleic acid equivalents. Fatty Acid are determined to estimate the nutritive value of fats. The result represents same quality of fat substance and shows the standard limit of Pharmacopoeia standard for Ayurvedic Formulation, CCRAS.

Test for Heavy Metals[21]: The quantity of specific potentially toxic metals in ayurvedia, unani/siddaand food stuffs are measured in the Heavy metals test. These heavy metals are lead, cadium, mercury and arsenic. Wet digestion procedure was used for detecting heavy metals. The metal content of ghrita will be calculated according to the following equation by AAC in ppm unit. Hinguvadhighrita sample were free from heavy metals and it was safe for therapeutic purpose.

Test for Afflatoxin[22]: Afflatoxin are procedued by fungus named Aspergillus flavus. These are closely related to secondary metabolism shows to be mycotoxin. These are four types of B1, B2, G1, G2. The residue of Aflatoxin is highly toxic and causes carcinogenicity. Aflatoxin are visualizing and easily separates by the application of thin layer chromatographic system with detection at uv254nm and 366nm. The result for Afflatoxin shows that HinguvadhiGhrita were free from Afflatoxins and it was safe for therapeutic purpose.

Table 3: Showing results of Physio-chemical parameters of HinguvadhiGhrita

S.no	Parameter	Results
1	Refractive Index	1.46117
2	Specific Gravity	0.9176g
3	Rancidity Test	Positive(oxidized)
4	Acid Value (w/w%)	2.62
5	Saponification value	228.36
6	Iodine Value	30.41
7	PH Value	5.00
8	Loss of Drying(w/w%)	0.31
9	Peroxide value(w/w%)	Nil
10	Total Fat content (%)	99.46
11	Total Fatty acids (w/w%)	1.31
12	Heavy metals • Lead (pb) • Cadmium (cd) • Arsenic (As) • Mercury (Hg)	 BLQ BLQ BLQ BLQ
13	Total Aflatoxins • Aflatoxins B1 • Aflatoxins B2 • Aflatoxins G1 • Aflatoxins G2	 BLQ BLQ BLQ BLQ

Microbiological Analysis[23]:

Total bacterial count: The most important test to evaluate microbial contamination in Ayurvedic Formulation and raw material is Total aerobic bacterial count.

Total fungal count: The most important test to evaluate fungal contamination in herbal formulation and raw material is Total Yeast and mould count.

The result of microbial analysis of HinguvadhiGhrita were found quite similar and under the standard limit of Pharmacopoeia standard for Ayurvedic formulation, CCRAS.

Table 4: Showing results of Microbiological Analysis of HinguvadhiGhrita

S.No	Microbial analysis	Result
1	Total microbial plate count	$<10^1$
2	Total yeast and mould count	$<10^1$
3	Escherichia coli	Absent
4	Salmonella spp.	Absent
5	Staphylococcus aureus	Absent
6	Pseudomonas aeruginosa	Absent

Phytochemical Analysis[24-27]:

To assess the phytochemical analysis, the plant extracts in ethanolic solution. The different active principles of Hinguvadhighrita subjected to qualitative tests to detects the various phytoconstituents as carbohydrates, lipids, alkaloids, terpenoids, tannis, protein etc

Hinguvadhighrita were dissolved separately in 5ml of alcohol and filtered. The presence of carbohydrates was tested by the filtrates[28].

(i) **Benedict Test:** Benedict reagent was subjected to filtrate and then heated on water bath, an orange red color is formed and precipitated indicating the presence of reducing sugar. The result show negative.

(ii) **Detection of protein and Amino acids as in Ninhydrin Test:** 2ml of Ninhydrin's reagent were treated with alcoholic extracts. The deep blue color (Ruhemann's purple) while heating indicating the presence of proteins. The results show negative.

(iii) **Detection of Alkaloids:** The alkaloid reagent was treated with the filtrated, by dissolving the extract individually in dilute HCl and filtered.

a) **Hagers Test:** Formed yellow precipitate indicating the presence of Alkaloids. The result show positive.

b) **Foam Test:** Formation of Persistent froth indicating the presence of saponin glycosides.

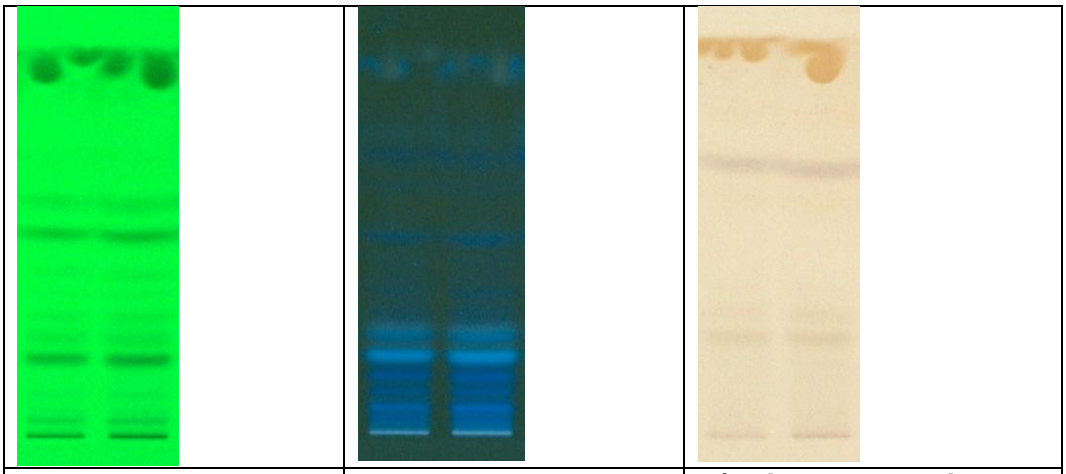
(iv) **Detection of Phenol and Tannis (Ferric Chloride Test):** Appearance of either green, orange, blue or purple-red color shows the test is positive.

(v) **Detection of Terpenoids:** Greyish color indicates the presence of terpenoids.

HPTLC[29]: The world Health Assembly has highlighted the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standard. It is the cardinal responsibility of the regulatory authorities to ensure that the herbal medicine is pure, safe, potent and efficacious. The scientific process of identifying different classes of phytoconstituents present in various parts is said to done by Phytochemical screening. To identify the phytochemical constituents by showing an electronic image of chromatographic fingerprint and a histogram to detect the existence of marker compound in a plant sample is known as High performance thin layer Chromatography (HPTLC).

HPTLC analysis of Hinguvadhighrita methanolic extract was performed for its phytochemical profiling. The pharmacological value of the Hinguvadhighrita is determined by the biological active compound present in it. Thin layer Chromatography profile of ghritam (Hexane Extract) was developed by using Hexane and Ethyl acetate in 7:3 ratio as solvent system 1ml of Hinguvadhighrita, dissolved in 10ml of chloroform and made up to 5ml in volumetric flask. The sample solution is 4, 6 µl of sample was applied on E.Merckaluminuim plate pre-coated with silica gel $60F_{254}$ of using CAMAG automatic sample applicator. The plate developed in Hexane: Ethyl acetate (7:3) up to 90mm and dried. The plate was observed through CAMAG TLC visualizer under UV-254nm and 366nm and photos were taken. Finally, the plate was dipped in vanillin sulphuric acid reagent and placed in hot air oven at 105 degree C until colour of spots were appeared and photo was documented. The scanning data confirms the chemical nature and distribution pattern in specified mobile phase by showing different spots. The ethanolic extracts shows spots under uv 254nm and 366nm which shows colored spots. To visualize the spots, derivatization is done using vanillin sulphuric acid reagent. It confirms the presence of higher alcohols, phenols and essential oils. Rf value indicates the position at which a substance is in the chromatogram. It is calculated by dividing the distance travelled by solute and solvent. The presence of black color spots with Rf value 0.23,0.29 and blue color spots with Rf value 0.63, organe color spot with Rf value 0.91 shows the presence of secondary metabolites which could be compared with standard for identification.

HPTLC fingerprint profile of DTL-2401782



UV-254	UV-366	After derivatization with VSR
Ethanol extract (Track-1 &2)-4, 6 µl		
Mobile phase: Hexane:Ethyl acetate (7:3)		

Fig.3: Showing the result of HPTLC of HinguvadhiGhrita

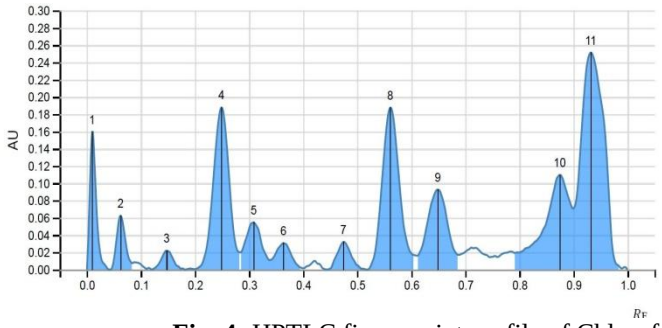
TLC Methodology

1ml of sample, dissolved in 10 ml of Chloroform and made up to 5 ml in volumetric flask. The sample solution is 4, 6 µl of sample was applied on E. Merck aluminuim plate pre-coated with silica gel $60F_{254}$ of using CAMAG automatic sample applicator. The plate developed in Hexane : Ethyl acetate (7:3)upto 90 mm and dried. The plate was observerd through CAMAG TLC Visualizer under UV-254 mm and 366mm photos was taken. Finally, the plate was dipped in vanillin sulphuric acid reagent and heated in hot air oven at 105^{o}C until colour of spots were appeared and photo was documented.

Table 5: Rf values

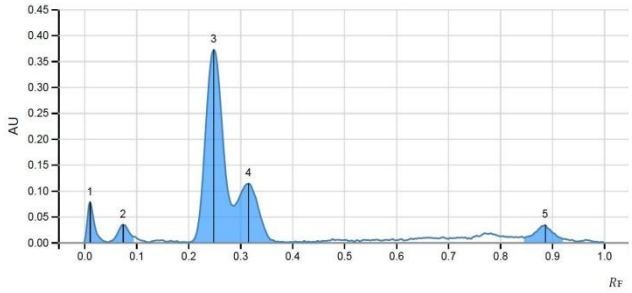
Sample name/Track no.	**UV-254**		**UV-366**		**Derivatised with vanillin sulfuric acid**	
Track-1	**Rf**	**Color**	**Rf**	**Color**	**Rf**	**Color**

	0.04, 0.11, 0.18, 0.23, 0.29, 0.48, 0.57, 0.87	Green	0.03, 0.06, 0.1, 0.14, 0.18, 0.23, 0.25, 0.47, 0.66, 0.86	Blue	0.23, 0.29	Black
					0.63	Blue
					0.91	Orange



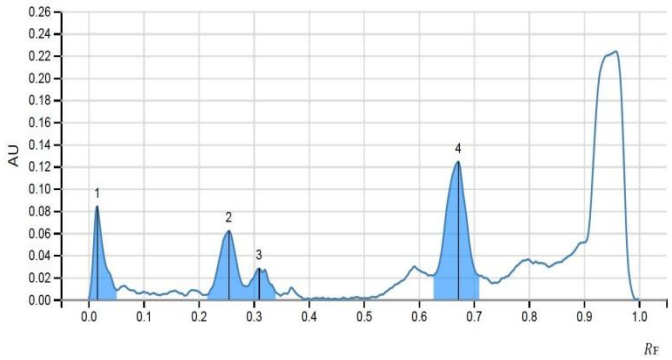
Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.010	0.1596	13.40	0.041	0.0000	0.00217	4.96
2	0.043	0.0000	0.062	0.0625	5.25	0.084	0.0075	0.00119	2.72
3	0.121	0.0000	0.148	0.0220	1.85	0.174	0.0011	0.00056	1.27
4	0.199	0.0004	0.249	0.1878	15.77	0.283	0.0200	0.00640	14.60
5	0.284	0.0199	0.309	0.0548	4.60	0.338	0.0111	0.00190	4.33
6	0.338	0.0111	0.363	0.0308	2.58	0.399	0.0016	0.00105	2.39
7	0.443	0.0000	0.474	0.0321	2.69	0.507	0.0012	0.00094	2.14
8	0.518	0.0000	0.561	0.1876	15.75	0.609	0.0163	0.00699	15.94
9	0.611	0.0161	0.649	0.0927	7.78	0.688	0.0170	0.00411	9.37
10	0.791	0.0193	0.874	0.1098	9.22	0.899	0.0709	0.00613	13.97
11	0.899	0.0709	0.932	0.2514	21.11	0.988	0.0014	0.01241	28.31

Fig. 4: HPTLC finger print profile of Chloroform extract track-1 at UV-254 nm



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.011	0.0774	12.28	0.045	0.0000	0.00119	5.16
2	0.045	0.0000	0.074	0.0340	5.40	0.096	0.0108	0.00088	3.81
3	0.200	0.0000	0.249	0.3716	58.99	0.287	0.0705	0.01434	62.26
4	0.288	0.0705	0.316	0.1135	18.01	0.370	0.0011	0.00521	22.61
5	0.846	0.0089	0.887	0.0334	5.31	0.922	0.0065	0.00142	6.16

Fig. 5: HPTLC finger print profile of chloroform extract track-1 at UV-366 nm



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.016	0.0839	28.13	0.051	0.0085	0.00187	17.00
2	0.209	0.0040	0.255	0.0619	20.78	0.285	0.0119	0.00231	21.02
3	0.287	0.0118	0.310	0.0280	9.39	0.349	0.0033	0.00107	9.72
4	0.626	0.0216	0.672	0.1243	41.71	0.711	0.0212	0.00574	52.26

Fig. 6: HPTLC finger print profile of chloroform extract track-1 at UV-540 nm

DISCUSSION

The therapeutic efficacy of the formulated medicine is completely depending on the quality of the raw drugs used in it. In response to the need of international harmonization in quality control testing of medicinal plant materials, this research has been done. The resultof the prepared formulation always depends on the drugs which meet the standards mentioned in API. The preliminary phytochemical screening is the qualitative test done for the detection of various Phyto-constituents that are responsible for the medicinal properties of drug.Phytochemical screening of Hinguvadhighrita shows the presence of proteins, alkaloids, saponin glycosides, terpenoids. Hinguvadhighrita has many therapeutic uses such as indigestion, pain, constipation, abdominal distention, rheumatoid arthritis, low back pain, appetizer, malabsorption syndrome etc. literature shows that rheumatoid arthritis is a chronic inflammatory auto immune disorder. Alkaloids present in hinguvadhighrita exhibit anti-proliferation, anti-bacterial, anti-viral, insecticidal and anti-metastatic effect. Saponin glycoside helps in prevention and treatment of various diseases such as obesity, vascular disease, cancer, decrease blood lipids. Pharmacological activities of Terpenoids shows anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, hypoglycemic activites and anti-cancer activites. Hinguvadhighrita helps in reducing and breaking the pathogens of rheumatoid arthritis by the action of anti proliferativeactivity of alkaloids, anti-inflammatory properties of saponin and Terpenoids.

CONCLUSION

The purpose of standardization of medicinal plants is to ensure therapeutic efficacy. The qualities of drug used in Hinguvadhighrita are equivalent as per the reference of API. The authenticity of drug is proven by the parameters, physico chemical analysis, phytochemical analysis. HPTLC profile of Hinguvadhighrita is considered as a prior tool authenticating the genuineness of HinguvadhiGhrita.

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