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A COMPARATIVE PHARMACEUTICO-ANALYTICAL STUDY OF GUDAPIPPALI GHRITAM BY USING NAVEENA AND PURANA PIPPALI

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ABSTRACT

Ghrita kalpana is a kind of formulation which is processed in a manner that both lipid and water soluble active principle of the drug are transferred into *ghrita. Gudapippali ghrita* is a formulation prepared by using *pippali, murchchita ghrita (*ghee*) godugdha* (cow's milk) and *guda* (jaggery). It is refer under *Amlapitta and Parinam-sula*[1]. Analytical study provides us the objective parameters for standardization. Analytical study for the standardization of Gudapippali ghrita was carried out on the basis of organoleptic test and modern parameters that is physio chemical properties such as Specific gravity, Refractive index, Rancidity etc. Chromatological techniques like high performance thin layer chromatography (HPTLC) and Gas chromatography and Mass spectrometry (GCMS) was carried out. As far as no previous comparative analytical study was carried out for the above said formulation, the study was taken up to standardise the product called *Gudapippali Ghritam*[2]. Hence it is comparative pharmaceutical and analytical study of *naveena* (new-dried) and *purana* (one year old –dried), which also help to compare any similarity and difference in samples.

Keywords: *Murchchita ghrita***,** Analytical study, HPTLC, GCMS

INTRODUCTION

'*Ayurveda*' is one of the most ancient systems of medicine, which has its own root in *Veda* and is an evolved system of health science based on its unique and original concepts and fundamental principles.

Bhaishajya kalpana is a broad subject including more relevant topics than modern pharmaceutical science. Only the method of Preparation, ingredients and some other related portions are included in modern pharmacy as it is a separate well developed wing. By applying these pharmaceutical techniques, number of *Kalpana's* were developed, one of them is *sneha kalpana*. The main advantage of *sneha kalpana* is that, *jala* (water) is also used in the formulation so both water soluble and fat soluble substances can be absorbed simultaneously.

Standardization of drug means confirmation of its identity, quality and purity throughout all phases of its cycle i.e. preparation, shelf-life, storage, distribution, and use by various parameters. The ayurvedic system of medicine is on the way to regain its past glory because of its preventive as well as curative nature and holistic approach with legal application of Good Agricultural and Collection Process (GACP),Good Manufacturing process (GMP),Good Laboratory practice(GLP) in *Ayurveda* pharmacy. These submissions give standardization of *ayurvedic* medicines which results in validation of evidence best clinical effect as described in *ayurvedic* texts. However efficient and accurate the physician is in his/her diagnosis and prescribes correct medicine it may not help the patient if the medicines of good qualities are not available. As we all know in our *Ayurvedic* system of medicines drug standardization of *Ayurvedic* formulation is a big challenge. Clear cut guidelines have not been developed so far, hence the above study is planned.

Pharmaceutico analytical standardization of *Gudapippali ghrita* with special reference to comparative study with *naveena* (new –dried) and *purana (*old) *pippali* has not been carried out. *Gudapippali ghrita* was prepared in two samples by using *jala* (water) and *godugdha* (cow's milk) as *Drava dravya*, *pippali* (new and old) and *guda* as *kalka* and *Murchchita ghrita.*

MATERIALS AND METHODS

Raw materials such as *purana* (old 1 year stored) and *naveena* (new-dried) *pippali, Godugdha* (cow's milk*), Goghrita* (ghee) and *Guda* (jaggery) were procured from reliable source (Anamaya drugs, Udupi). Raw material analysis like Loss on drying, Ash value, Extractive and purity test like Mineral oil, Methanin yellow etc. were performed. Preparation was carried out in pharmacy section of PG Department of *Rasashastra* and *Bhaishajya kalpana* MIAMS Manipal.

PROCEDURE

In preparation of *Naveen gudapippali ghrita*, *sneha* was processed along with *naveena pippali* (new-dried*) kalka, Jala* (water), *Godugdha* (cow's milk) until the *sneha siddhi lakshanas* are obtained. After filtering *ghrita,* required quantity *guda* was added. Same procedure was followed for preparation of *Purana gudapippali ghrita* where *pippali* fruit was taken 1 year old stored and *guda paka* was separately done. The change in preparation was done because while preparation of sample of *Naveena gudapippali ghrita, guda* (jaggery) was added at end to the product in case it was not uniformly mix with product and seen settled at bottom hence to overcome this, *guda* (jaggery) was added after doing *guda paka* in preparation of *Purana pippali ghrita.* Raw material used in ratio as *Godugdha* (cow's milk)-4 parts, *Goghrita* (Ghee)-1part, *Jala* (water)-4parts, *Pippali kalka+guda* (jaggery)-2/8 parts. Temperature was 80-90.2°C. Total time duration 6 hour 30 minutes approximately for each sample, obtained quantity 250ml of each sample, Time of preparation September-October month.

ANALYTICAL STUDY

To assess the quality of prepared ghrita was subjected to both classical and modern parameters

A. Classical parameters

Parameters like *Varna, Gandha* etc. were carried out for *ghrita* in *Rasashastra* and *Bhaishajya kalpana* Department MIAMS Manipal

B. Physico-chemical parameters

Physical test like Organoleptic, Acid value, Specific gravity etc. were conducted in QC lab of MIAMS Manipal, Peroxide value conducted in MCOPS MAHE Manipal, HPTLC, GC-MS and Viscosity were conducted in CARe Keralam, Koratty,Kerala.

1) Loss on drying (LOD)[4]

Petri dish was cleaned with distilled water and dried in oven at 105°C for 2hours. 5g of ghrita was taken in Petri dish. It was kept in the oven for drying at 105°C for 5 hrs. After cooling the weight was recorded. It was again subjected to drying for an hour and then weight was noted. This procedure was repeated till two consequent weights which are same were obtained. The weight loss is calculated and expressed as %w/w.

2) Specific gravity[5]

A 25 ml capacity Pycnometer was taken and its weight was noted. It was then filled with the ghrita, weight was taken at 40°C.Then it was rinsed and filled with distilled water, weight was noted at 40°C.

The weight of ghrita and distilled water was calculated. Then the Specific gravity was determined by dividing the weight of the ghrita by the weight of the water.

3) Refractive Index[6]

Make refractometer in proper caliberation by means of suitable standard. Place a few drops of ghrita on the lower prism of refractometer. Close prism tightly and allow a short time for the ghrita to come to the temperature of the instrument and then read the refractive index. The ghrita should be removed after each examination with a small swab of cotton saturated with a suitable solution such as toluene etc.

4) Viscosity[7]

Clean the viscometer with water, rinse with acetone and dry it. Using a clean pipette, transfer a known volume of water in to the wider limb. Allow the viscometer to stand for some time so that the liquid attains the temperature of the bath. Suck the water above the upper mark of the viscometer. Allow it to flow freely through the capillary tube. When the level of water just crosses upper mark, start a stop clock when the water just crosses the lower mark. Note the time of flow in seconds (tl). Repeat the experiment with exactly the same volume of the ghrita.

5) Acid value[8]

1gm of ghrita was weighed and placed in a conical flask, to it add 50ml of mixture of equal volumes of alcohol and solvent ether, to that add 1ml of solution of phenolphthalein indicator. Afterward titrate with 0.1N potassium hydroxide, shaking constantly until a pink colour persist. Note the number of ml required. Calculate the value using formula.

6) Iodine value[9]

Place accurately weighed ghrita in a dry iodine flask**.** Add 10ml of carbon tetrachloride & dissolve to it add 20 ml of Wij's solution shake & keep it in a dark place for 30 min in a temperature between 15°C to 25°C afterwards add 15 ml of potassium iodide and 100 ml of distilled water. Shake and titrate with 0.1N sodium thiosulphate using solution of starch as indicator. Note the no. of ml of 0.1N sodium thiosulphate required.

7) Saponification value[10]

Weighed 2g of ghrita into a 250 ml round bottom flask fitted with a reflux condenser. Add 25ml of 0.5M Alcoholic Potash. Keep on a water bath for 1 hour. After cooling add 1ml of Phenolphthalein solution and titrate immediately with 0.5M Hydrochloric acid. Note the value.

8) Peroxide value[11]

1g of ghrita was weighed and placed in a clean conical flask, add 3 volume of glacial acetic acid and 2 volume of chloroform and 0.5ml volume of potassium iodide solution to it. Allow to stand for exactly 1 minute, with occasional shaking, add 30ml of water and titrate gradually with continuous and vigorous shaking with 0.01M sodium thiosulphate until yellow colour almost disappears. Add 0.5ml of starch solution and continue titration, shaking vigorously until the blue colour just disappears. Calculate the value by using formula.

9) Rancidity test (Krei's test) [12]

Mix 1 ml of melted ghrita with 1 ml of conc. hydrochloric acid in a test tube. Add 1 ml of one percent solution of phloroglucinol in diethyl ether and mix thoroughly with the ghrita and acid mixture. A pink colour formation indicates that the ghrita is slightly oxidized while a red colour indicates that the ghrita is definitely oxidized.

HPTLC (High performance thin layer chromatography)[13]

Sample preparation: 10 ml of sample was partitioned with 20 ml of methanol and methanol soluble portion was used for HPTLC 3, 6 and 9µl of the sample was applied on a precoated silica gelF254 on an aluminum plate to a band width of 8mm using Linomat 5TLC applicator. The plate was developed in Toluene-Ethyl acetate (9:1) and the developed plates were visualized under UV 254 and 366nm.R_f colour of the spots and densitometer scan were recorded.

GCMS (gas chromatography mass spectrometer) [14]

Gas chromatography is an analytical technique used for separation of thermally stable and volatile substances. It is widely used because of its speed, resolving power and sensitivity. In this mobile phase used is gas and the stationary phase used may be a solid or liquid. This parameter was used to find the fatty acid

groups present in the sample with Methylated fatty acid marker as the standard. Gas chromatography analysis was performed by Agilent GCMS 5975 C with FID using HP-5 capillary column. GC-MS analysis was performed using a Shimadzu QP 5050A mass spectrometer coupled with a Shimadzu 17A gas chromatograph fitted with a split-split less injector and a DB-5 fused silica capillary column (30m X 0.25mm i. d., 0.25 µm film thicknesses). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 250ºC, and the split ratio was 40:1.Oven temperature programming was done from 50 to 280ºC, at 10 0 C/min and it was kept at 280°C for 5 min. Interface temperature was kept at 220ºC. Source temperature was 1800C.

RESULTS

RAW DRUG ANALYSIS

Purity test for *Ghrita*:-

Table 1: showing purity test for *ghrita*

Test	Observation	Inference
Coal tar dyes	No pink or crimson colour	Absent
Mineral oil	No turbidity	Absent
Vanaspathy/margarine	No appearance of crimson colour in lower part	Absent
Mashed potatoes, sweet potatoes and other starches	No brownish to blue colour	Absent
Furfural test	No crimson colour	Absent

Specific gravity - 0.923g/ml
Refractive index - 1.4526
Acid value - 0.332
Iodine value - 35.30
Saponification value - 213.42
LOD - 0.36

Purity test for *Godugdha*:-

Table 2: Showing purity test for *godugdha*

Test	Observation	Inference
Water	Left white trail behind after flow and the flow was slow	Absent
Starch	No blue colour, yellow colour was seen	Absent
Glucose	Strip did not turn blue to green	Absent
Sugars	There is no red discolouration of milk (it was off white colour)	Absent
Vanaspati	There was no red colouration	Absent
Formalin	There was no violet or blue ring formation, brown ring was seen.	Absent
Detergent	No lather was seen	Absent
Synthetic milk	No bitter taste, soapy feeling on rubbing between the fingers was not seen	Absent

Purity test for *Guda*:-

Table 3: showing purity test for *guda*

Test	Observation	Inference
Sodium bicarbonate	No effervescence	Absent
Metanil yellow colour	Light pink colouration	Present
Washing soda	No effervescence	Absent
Chalk powder	No settled chalk powder	Absent

Estimation test of sugars for *Guda*:-

Table 4: Showing estimation of sugars for *guda*

Sample	Total sugars	Reducing sugars	Sucrose%
Guda	91.81%	23.48%	64.91%

Quality test for *pippali*:-

Table 5: Showing purity test for *pippali (naveena* and *purana*)

Drug Name	Total Ash	Water soluble Ex-tractive	Alcohol soluble Ex-tractive	Acid insoluble Ash	LOD
Naveena Pippali	6.79%	68.2%	43.1%	0.34%	10.2%
Purana pippali	7.43%	54.4%	36.4%	0.46%	7.8%
API standards	Not more than 7%	Not less than 7%	Not less than 5%	Not more than 0.5%	

Preliminary phyto-chemical screening of *Pippali* (Aqueous extract):-

Table 6: Showing phyto chemical screening of *pippali (naveena* and *purana*) – Aqueous extract

Sr No	Test		Result for *naveena(new-dried) pippali*	Result for *purana(old) pippali*
1	Alkaloids	Mayer's test	+ve	+ve
		Hager's test	+ve	+ve

Preliminary phyto-chemical screening of *Pippali* (Methanol extract):-

Table 7: Showing phyto chemical screening of *pippali (naveena* and *purana*) – Methanol extract

Sr No	Test		Result for *naveena (new-dried) pippali*	Result for *purana (old)pippali*
1	Alkaloids	Mayer's test	+ve	+ve
		Hager's test	+ve	+ve

FINISHED PRODUCT ANALYSIS

Table 1: Organoleptic characters

Parameters	*Naveena gudapippali* (new-dried)	*Puranagudapippali* (old)
Colour	Golden yellow	Green
Odour	Smell of ghee and *pippali*	Smell of ghee, *pippali* and sweet smell of *guda*
Taste	*Katu, tikta*	*Tikta,* slight *madhura*
Consistency	Oily, liquid	Liquid, oily

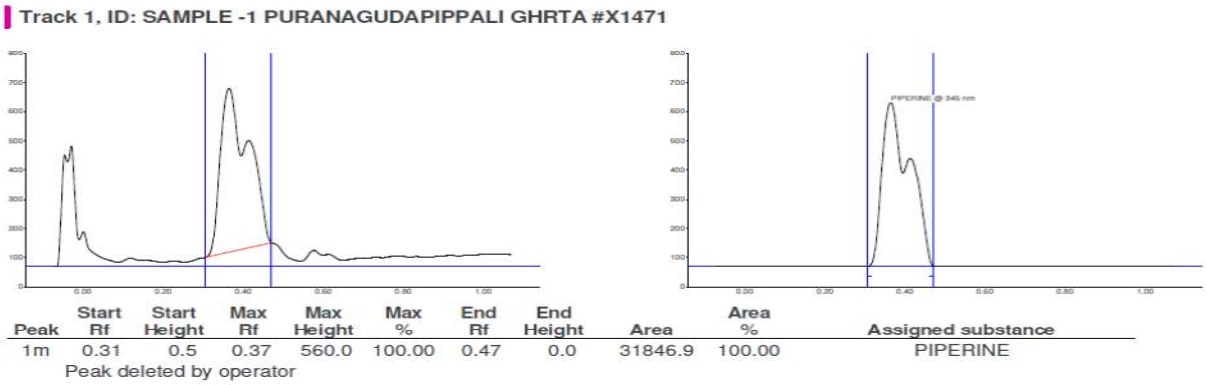
Table 2: Physico-chemical parameters

Parameters	*Naveena gudapippali* (new-dried)	*Puranagudapippali* (old)
Loss on drying	0.28	0.17
Specific gravity	0.91	0.94
Refractive Index	1.452	1.460
Acid value	2.33	2.43
Iodine value	29.08	24.48
Saponification value	229.9	187.3
Viscosity	80610 cps	82680 cps
Peroxide Value	2.3292	16.9321
Rancidity	Not Oxidized	Not Oxidized

Table 3: HPTLC Results

Instrumental methods	*Naveena guda pippali* (new-dried)	*Purana guda pippali* (old)
HPTLC Analysis	0.11%	0.069%

Figure 1: Graph of PGPG (*purana gudapippali ghrita)*



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1m	0.31	0.5	0.37	560.0	100.00	0.47	0.0	31846.9	100.00	PIPERINE

Peak deleted by operator

Figure 2: HPTLC photo documentation of sample of piperine fraction *Puranagudapippali Ghrita*

At 254nm At 366nm

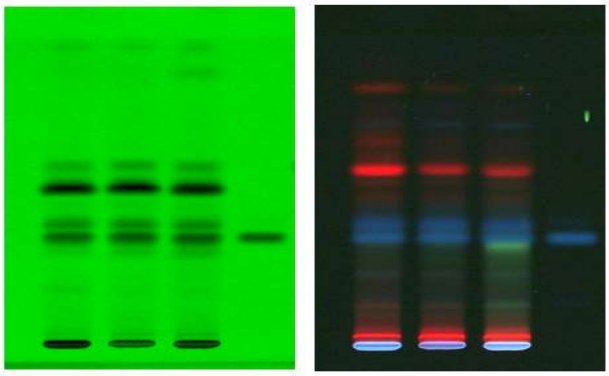
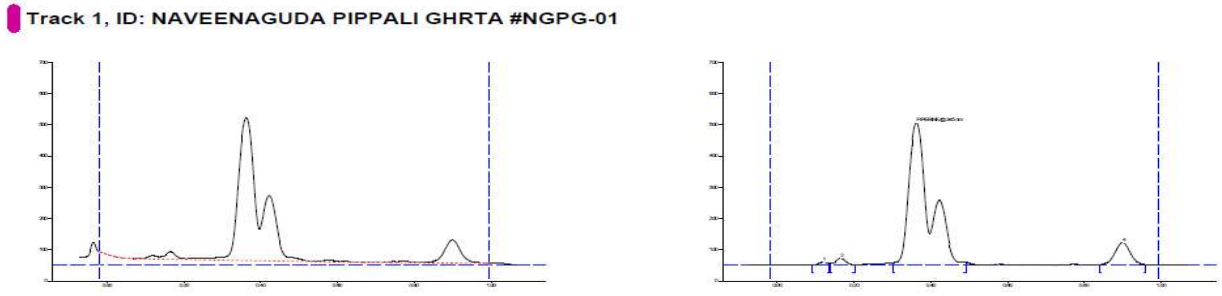


Figure 3: Graph of NGPG (*naveena gudapippali ghrita)*



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	1.3	0.12	11.0	1.94	0.14	6.4	214.3	0.89	unknown *
2	0.14	6.5	0.17	23.5	4.16	0.20	2.9	549.4	2.29	unknown *
3m	0.30	8.5	0.36	457.3	80.89	0.49	9.8	20806.4	86.54	PIPERINE
Peak deleted by operator										
4	0.84	1.4	0.90	73.6	13.01	0.96	2.9	2472.0	10.28	unknown *

Figure 4: HPTLC photo documentation of sample of piperine fraction *Naveena gudapippali Ghrita*

At 254nm At 366nm

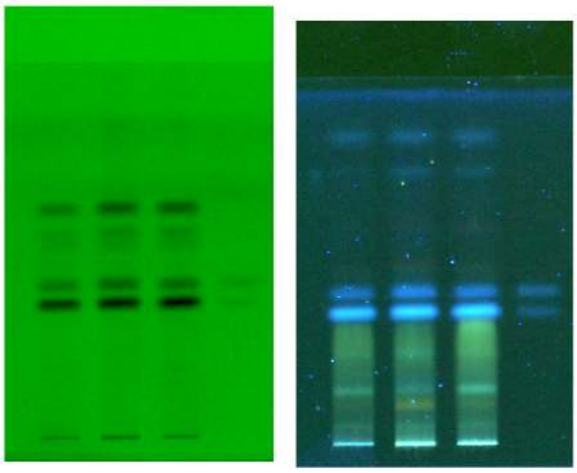


Table 4: Showing GCMS Values for NGPG (*naveena gudapippali ghrita)*

PARAMETERS	
FATTY ACID PROFILE	**RESULT**
Butyric acid	0.65%
Caproic acid	0.83%
Caprylic acid	0.72%
Capric acid	1.98%
Lauric acid	2.8%
Myristic acid	11.31%
Palmitic acid	**37.82%**
Palmitoleic acid	1.98%
Stearic acid	13.01%
Trans-vaccenic acid	2.76%
Elaidic acidacid	**23.22%**
Linolenic acid	2.92%

Table 5: Showing GCMS Values for PGPG (*purana gudapippali ghrita)*

PARAMETERS	
FATTY ACID PROFILE	**RESULT**
Butanoic acid	0.52%
Caproic acid	0.62%
Caprylic acid	0.61%
Capric acid	1.67%
4-Decanoic acid	0.16%
Undecanoic acid	0.03%
Lauric acid	2.47%
Tridecanoic acid	0.10%
Myristic acid	**10.79%**
Methyl Myristoleate	1.01%
Pentadecanoic acid	1.19%
Palmitic acid	**34.67%**
Palmitoleic acid	2.14%
Margaric acid	0.77%
Cis 10 Heptadecanoic acid	0.44%
Stearic acid	**12.90%**
Elaidic acid/Oleic acid	**22.05%**
11-Octadecnoic acid	3.13%
Linoleic acid	2.99%
12,15 Octadecadienoic acid	0.11%
Nonadecanoic acid	0.14%
Cis 10 Nonadecanoic acid	0.20%
Linolenic acid	0.27%
Arachidic acid	0.21%
Eicosenoic acid	0.24%
8,11,14 Eicosatrienoate acid	0.09%
Arachidonic acid	0.14%
Behenic acid	0.19%
Lignoceric acid	0.15%

DISCUSSION

An attempt was made to prepare two samples of *Gudapippali ghrita* with *naveena* and *purana pippali* fruit for the comparative evaluation and the outcome was highly successful. In organoleptic characters, *Gudapippali ghrita* prepared by using *naveena pippali* was golden yellow in Colour but the sample prepared by using *purana pippali* was greenish in Colour. Sample of *Gudapippali ghrita* prepared by *purana pippali* exhibited pleasant ghee smell along with sweet smell of *guda* whereas samples of *Gudapippali ghrita* prepared by *naveena pippali* exhibited pleasant ghee smell along with smell of *pippali*. Raw drug analysis was carried out for *goghrita* (ghee), *godugdha* (cow's milk), *guda* and *pippali* hence there was no presence of any adulterants. The instrumental analysis such like HPTLC and GC-MS was carried out for comparison with the standard value and fingerprints of *pippali*. In the case of *Gudapippali ghrita* samples prepared by using *Naveena pippali* samples showed average moisture content of 0.053% w/w which slightly increases to an average value of 0.23% w/w. Specific gravity of both the samples were almost similar. Re-

fractive index of both the samples showed closely similar values. Viscosity of *Gudapippali guda* sample prepared by using *Naveen pippali* was quite high when compared with *Gudapippali ghrita* prepared by using *purana pippali*. There was a mild and progressive increase of acid values as the time laps which indicates generation of more fatty acids along with time. However there was no appreciable difference in acid value in between the samples. Comparatively *Naveena gudapippali ghrita* samples showed lesser iodine value indicating their better stability. NGPG *(naveena gudapippali ghrita*) sample showed comparatively higher amount of piperine with an average of 0.1266% and PGPG (*purana gudapippali ghrita*) sample showed piperine quantity of 0.06833% as on average. These values were compared with standard of *pippali*. However GC-MS graph with specific peaks can be used as finger prints of fatty acids for the standardization of *Gudapippali ghrita.*

CONCLUSION

The comparative study showed difference organoleptically and in iodine value. The study findings confirm the ingredients present in *Gudapippali ghrita* and identified phytochemical component like piperine. *Gudapippali ghrita* is an easier preparation which is widely used for its efficacy in *Amlapitta* and a safer medicine to prescribe for any period of time and any age group of patients. Hence the study efforts to standardize the medicine have been done. Further study like real time stability data and clinical study can be carried out.

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