



Research Article

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A COMPARATIVE PHYSICO-CHEMICAL ANALYSIS OF MURCCHITA GO- GHRUTA AND ASHTAMANGALA GHRUTA

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ABSTRACT

Ayurvedic medicines are compound formulations of natural origin. It is essential to maintain the analytical standards of each formulation for a quality production of these medicines. Ghruta Murcchana is a unique pharmaceutical process prior to the preparation of Ghruta formulations. Ashtamangala Ghruta is a formulation indicated for the treatment of all types of fever. Both Murcchita Go-Ghruta and Ashtamangala Ghruta are prepared according to the standard operative procedure mentioned in the classics. The present study was focused on analytical studies of Murcchita Go-ghruta and Ashtamangala Ghruta, which was carried out separately on the basis of classically illustrated organoleptic tests, modern physico-chemical parameters like Loss on Drying at 110°C, Refractive index at 40°C, Ester value, Saponification Value, Acid Value, Iodine Value, Peroxide Value and HPTLC etc. The present study revealed that Saponification value, Iodine Value, Acid value, Ester value, Specific gravity were higher in Ashtamangala Ghruta than in Murcchita Go-ghruta which suggests that more active constituents were present in Ashtamangala Ghruta. HPTLC showed that maximum number of spots in Ashtamangala Ghruta than in Murcchita Go-ghruta at UV 254 nm and UV 366 nm. The marker compounds found in HPTLC can be identified and used as referral standards in future studies. Hence the present work may be used for the quality assessment and standardization of Ashtamangala Ghruta.

Keywords: Ghruta, Ashtamangala Ghruta, Murcchana, Analytical, TLC, HPTLC.

INTRODUCTION

Ghruta Kalpana is a group of formulations which are processed in a manner that both lipid soluble and water soluble active principles of the drugs are transferred into Ghruta, which is used here as a base. Unlike other dosage forms, because of its lipophilic action, Ghruta Kalpana can cross the blood- brain- barrier delivering the active principles of the ingredients at the specific sites of their action¹. Ashtamangala Ghruta is one among them mentioned in Sahasrayoga, Ghrutha prakarana which is indicated in the treatment of all types of fever². It is an important formulation mentioned in other classics with different compositions for the treatment of various diseases. Ghruta Murcchana is a unique pharmaceutical procedure which is to be carried out on uncooked Ghruta before subjecting it to formulation. Due to Murcchana, Ghruta may become capable to receive more active principles during the subsequent preparation along with the active principles of Murcchana Dravyas. This process is referred as refining of ghee and is aimed at removing of free fatty acids, undesirable odor and moisture from Ghruta, hence alters its physical as well as chemical characteristics³. These physico-chemical changes occurring while processing the Ghruta with different pharmaceutical techniques can be better understood by the analytical parameters and chromatographical parameters. In the present study cow's ghee (Go-ghruta) was taken as the base for the preparation of samples as it is a good medium for absorption, transport and delivery of drug and as it contains long chain of polyunsaturated fatty

acids thereby increases the shelf life. The present work was focused on analytical study and HPTLC profile of Murcchita Goghruta and Ashtamangala ghruta.

Aims and objectives

- To analyze Murcchita Go-ghruta and Ashtamangala Ghruta physico-chemically
- To analyze Murcchita Go-ghruta and Ashtamangala Ghruta chromatographically.

MATERIALS AND METHODS

Source of data

Market sample of Go Ghruta was collected from a reputed and popular company. The certified raw drugs prescribed in the formulations were collected from Alva Pharmacy, Mijar, Moodabidri, Karnataka, India. All raw drugs were identified as genuine samples by the experts from Department of Dravyaguna, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India. Pharmaceutical study was carried out in the laboratory of P. G. Department of Rasashastra and Bhaishajya Kalpana, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India.

Method of collection of data

Two steps were involved in the procedure:-

Ghruta Murcchana

In the process of preparation of Ashtamangala Ghruta, the initial procedure was Ghruta Murcchana. It was carried

out as per Bhaishajya Ratnavali⁴. Ghruta murcchana was carried out by heating goghruta with the kalka prepared out of drugs like Hareetaki (*Terminalia chebula* Rex), Vibheetaki (*Chebulic myrobalan* Roxb), Amalaki (*Emblica officinalis* Gaertn), Musta (*Cyperus rotundus* Linn), Haridra (*Curcuma longa* Linn) and Nimbu Swarasa (*Citrus limon* Linn) and 4 parts of water, in moderate heat (around 110°C) till the sneha siddhi lakshanas were obtained. The process was completed in one day.

Preparation of Ashtamangala Ghruta

Ashtamangala Ghruta was prepared according to sneha kalpana procedure described in Sharangadhara Samhita⁵. There are eight ingredients namely Patola (*Tricosanthes dioica* Roxb), Sariva (*Hemidesmus indicus* R.Br), Musta (*Cyperus rotundus* Linn.), Yashtimadhu (*Glycyrrhiza glabra* Linn.), Katurohini (*Picrorrhiza kurruoa* Royle.ex.Benth.), Usheera (*Vetiveria zizanoids*), Chandana (*Santalum album*) and Pippali (*Piper longum* Linn). These drugs were mentioned for the preparation of both kashaya (as dravadravya) and kalka. First kashaya was prepared using same drugs mentioned above. Fine paste of these drugs added to heated Murcchita Ghruta and then kashaya was added and heated in mandagni (60-80°C). The heating was repeated for 12 days since majority of the ingredients are either roots, rhizomes or climbers.⁶ The process was completed on 12th day. The process repeated and thus two batches of Murcchita Goghruta and Ashtamangala Ghruta were prepared. Each time, observations were made at different stages, temperature, sneha siddhi lakshanas⁷, loss of percentage in the final product and duration of process were noted down.

Analytical Study

The organoleptic, physico-chemical parameters and HPTLC were processed in Murcchita Ghruta and Ashtamangala Ghruta.

Organoleptic characters

These are subjective parameters involved in testing of drugs by sense organs, includes color, odor, touch, taste and appearance.

Physico-chemical parameters

The various analytical tests conducted were Acid value, Saponification value, Iodine value, Loss on drying at 110°C, Ester value, Refractive index, TLC, HPTLC studies etc. in SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, India and Quality Testing Laboratory of Vaidyaratnam Oushadhasala Pvt. Ltd, Thrissur, Kerala, India as a part of this study. All the tests were done as per the standard pharmaceutical laboratory process given in Appendix 3 (Physical test determination) of the Ayurvedic Pharmacopeia of India⁸.

HPTLC study

For the HPTLC study, chloroform extract of unsaponifiable matter of Murcchita Go-ghruta and Ashtamangala ghruta were prepared and labeled as track 1 and 2. The HPTLC conditions were as below:
Stationary phase - Silica gel-G pre coated plate
Mobile phase – Toluene: Ethyl acetate (8:1)
Visualization – Under short (254 nm) and long (366 nm) U.V

RESULTS

Organoleptic properties

The Murcchita Go-ghruta was dark yellow in color and had characteristic ghee smell and aromatic odor of Haridra. Ashtamangala Ghruta was dark brownish yellow in color and had a fragrance during the preparation with strong bitter taste. The differences in the two batches were noted and showed in Table 1.

Table 1: Observations of organoleptic properties of Murcchita Goghruta and Ashtamangala Ghruta-Batch-I and Batch-II Analytical Parameters

Sample	Color	Odour	Taste	Appearance
Murcchita Ghruta-I	Light Yellow	Characteristic ghee smell	Characteristic ghee taste	Greasy
Ashtamangala Ghruta-I	Brownish Yellow, on cooling-Greenish yellow	Characteristic ghee smell	Bitter	Oily, viscous
Murcchita Ghruta-II	Dark Yellow	Characteristic ghee smell with aromatic odor of Haridra	Characteristic ghee taste with mild sweetness	Greasy
Ashtamangala Ghruta-II	Dark Brownish Yellow, On cooling-Dark greenish yellow	Characteristic ghee smell with fragrance of drugs	Strong Bitter	Oily, viscous

Results of physico-chemical parameters of Murcchita go-Ghruta and Ashtamangala Ghruta are showed in Table 2.

Table 2: Physico-chemical parameters of Murcchita Go-ghruta and Ashtamangala Ghruta Chromatographic findings

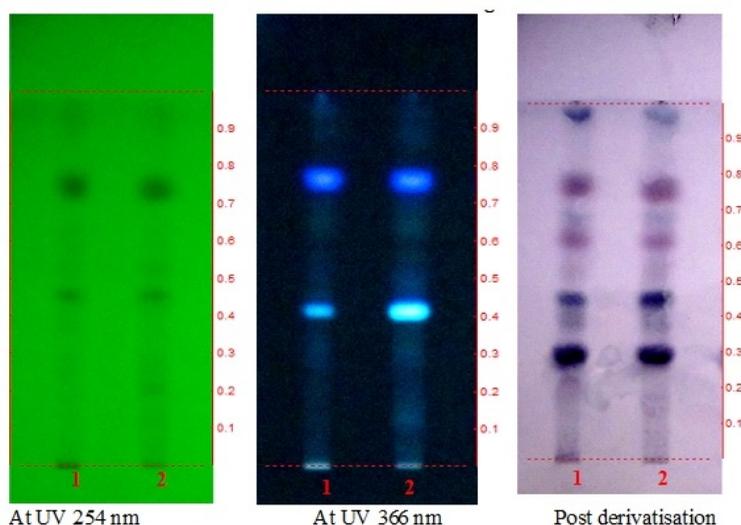
Parameters	Murcchita Go-Ghruta	Ashtamangala Ghruta
Loss on drying	0.254 %	0.242 %
Refractive index	1.45844	1.45844
Specific gravity	0.91533	0.91833
Viscosity	40 minutes/50 ml	39 minutes/50 ml
Saponification value	197.26 mg/ml	201.22 mg/ml
Iodine value	4.832 g/ml	5.253 g/ml
Acid value	0.871 g/ml	0.795 g/ml
Ester value	196.465 g/ml	200.349 g/ml
Unsaponifiable matter	0.6426 g	0.9219 g
Total fatty matter	99.357 m Eq/L	99.078 m Eq/L

The R_f values obtained were measured using scale showed in Table 3.

Table 3: R_f value of chloroform extract of unsaponifiable matter of Murcchita and Ashtamangala Ghruta

At UV 254 nm		At UV 366 nm		Post - derivatisation	
Murcchita Ghruta	Ashtamangala Ghruta	Murcchita Ghruta	Ashtamangala Ghruta	Murcchita Ghruta	Ashtamangala Ghruta
-	0.06 L Green	-	-	-	0.06 L Blue
-	0.13 L Green	-	0.13 F L Blue	-	0.13 L Blue
-	0.19 L Green	-	-	-	0.19 L Blue
0.24 L Green	-	-	-	0.24 Violet	-
0.25 L Green	0.25 L Green	-	-	-	-
0.29 L Green	0.29 L Green	-	-	0.29 violet	0.29 violet
-	-	-	-	0.38 L Blue	0.38 L Blue
-	-	0.42 F M Blue	0.42 F M Blue	-	-
0.45 Green	0.45 Green	-	-	0.45 Blue	0.45 Blue
0.53 L Green	0.53 L Green	-	-	-	-
-	-	-	0.57 F L Blue	-	-
-	-	-	-	0.62 Pink	0.62 Pink
-	-	0.65 F L Blue	0.65 F L Blue	-	-
-	-	-	-	0.68 Blue	0.68 Blue
0.75 Green	0.75 Green	-	-	-	-
-	-	0.77 F Blue	0.77 F Blue	0.77 Pink	0.77 Pink
-	-	0.88 F L Blue	0.88 F L Blue	-	-
-	-	0.88 F L Blue	0.88 F L Blue	-	-
-	-	0.95 F L Blue	0.95 F L Blue	0.95 Blue	0.95 Blue

D- Dark, L - Light, F- Fluorescent



Track 1: Chloroform extract of unsaponifiable matter of Murchita Ghruta 5 µl
 Track 2: Chloroform extract of unsaponifiable matter of Ashtamangala Ghruta 5 µl
 Solvent system – Toluene : Ethyl acetate (8: 1)

Figure 1: TLC Photodocumentation of chloroform extract of unsaponifiable matter of Murchita and Ashtamangala Ghruta

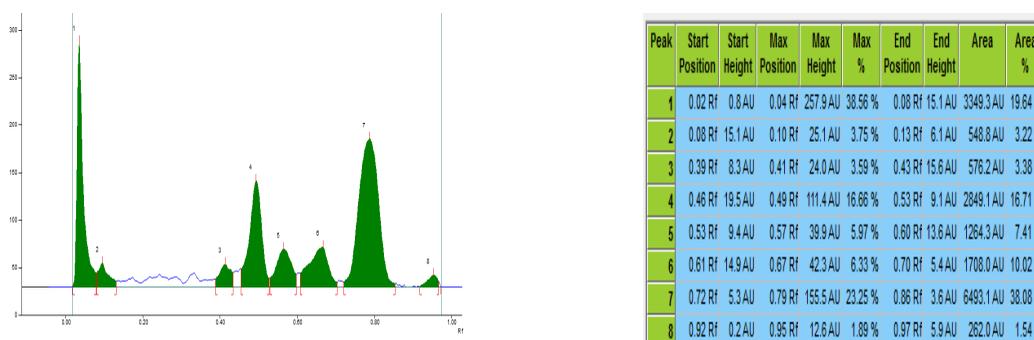


Figure 2: HPTLC Densitometric scan of chloroform extract of unsaponifiable matter of Murcchita Goghrruta at 254 nm

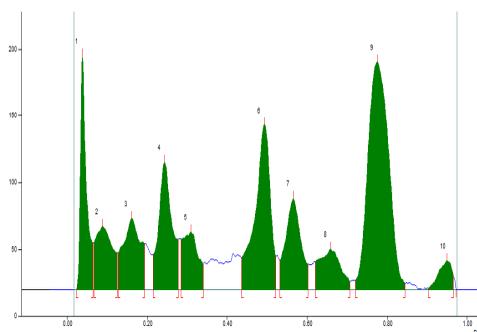


Figure 3: HPTLC Densitometric scan of chloroform extract of unsaponifiable matter of Ashtamangala Ghruta at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.1 AU	0.04 Rf	175.1 AU	21.16 %	0.07 Rf	35.0 AU	2144.4 AU	8.75 %
2	0.07 Rf	35.1 AU	0.09 Rf	47.1 AU	5.70 %	0.13 Rf	27.7 AU	1818.6 AU	6.61 %
3	0.13 Rf	27.4 AU	0.16 Rf	53.0 AU	6.41 %	0.19 Rf	34.3 AU	1859.3 AU	7.59 %
4	0.22 Rf	26.0 AU	0.24 Rf	95.1 AU	11.51 %	0.28 Rf	37.5 AU	2557.8 AU	10.44 %
5	0.29 Rf	37.6 AU	0.31 Rf	42.9 AU	5.19 %	0.34 Rf	19.5 AU	1366.5 AU	5.58 %
6	0.44 Rf	23.8 AU	0.49 Rf	123.3 AU	14.91 %	0.52 Rf	24.8 AU	3835.8 AU	15.65 %
7	0.53 Rf	21.9 AU	0.57 Rf	68.0 AU	8.23 %	0.60 Rf	19.2 AU	2089.5 AU	8.53 %
8	0.62 Rf	20.9 AU	0.66 Rf	30.1 AU	3.64 %	0.71 Rf	6.0 AU	1301.1 AU	5.31 %
9	0.72 Rf	6.8 AU	0.78 Rf	170.5 AU	20.62 %	0.85 Rf	4.8 AU	7161.0 AU	29.22 %
10	0.90 Rf	0.9 AU	0.95 Rf	21.5 AU	2.60 %	0.97 Rf	10.7 AU	570.0 AU	2.33 %

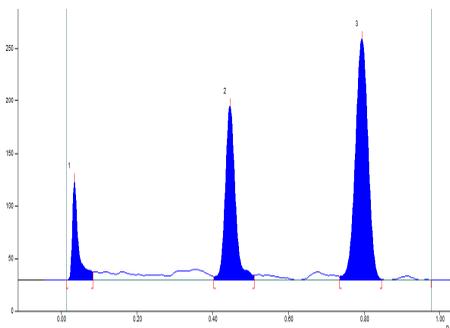


Figure 4: HPTLC Densitometric scan of chloroform extract of unsaponifiable matter of Murchita Ghruta at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.04 Rf	94.4 AU	19.25 %	0.08 Rf	8.0 AU	1235.4 AU	11.22 %
2	0.40 Rf	3.3 AU	0.45 Rf	166.1 AU	33.88 %	0.51 Rf	4.6 AU	3569.9 AU	32.43 %
3	0.74 Rf	3.5 AU	0.80 Rf	229.9 AU	46.88 %	0.85 Rf	0.9 AU	6201.9 AU	56.34 %

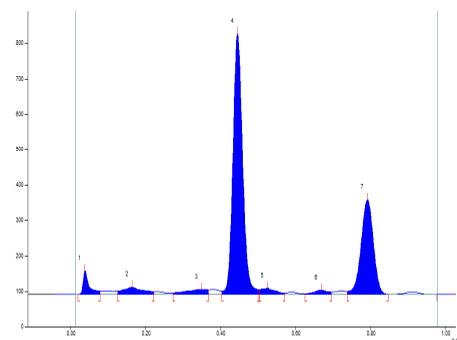


Figure 5: HPTLC Densitometric scan of chloroform extract of unsaponifiable matter of Ashtamangala Ghruta at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.3 AU	0.04 Rf	66.8 AU	5.89 %	0.08 Rf	9.9 AU	889.5 AU	3.42 %
2	0.13 Rf	9.1 AU	0.16 Rf	20.4 AU	1.80 %	0.22 Rf	7.7 AU	895.6 AU	3.45 %
3	0.27 Rf	4.4 AU	0.35 Rf	14.5 AU	1.28 %	0.37 Rf	12.6 AU	673.1 AU	2.59 %
4	0.40 Rf	8.8 AU	0.45 Rf	736.0 AU	64.83 %	0.50 Rf	13.5 AU	15495.3 AU	59.65 %
5	0.50 Rf	13.6 AU	0.53 Rf	18.4 AU	1.62 %	0.57 Rf	3.7 AU	551.4 AU	2.12 %
6	0.63 Rf	1.6 AU	0.67 Rf	12.0 AU	1.05 %	0.70 Rf	6.6 AU	367.8 AU	1.42 %
7	0.74 Rf	8.5 AU	0.79 Rf	267.1 AU	23.53 %	0.85 Rf	0.0 AU	7105.8 AU	27.35 %

HPTLC study was done just to obtain the fingerprints of the preparation and it was also carried out to get standard markers for the study. TLC photo documentation was done for the samples of Murchita Go-ghruta and Ashtamangala Ghruta as showed in Figure 1. HPTLC densitometric scan of chloroform extract of unsaponifiable matter of Murchita Goghruta at 254 nm showed 8 peaks which covered the area of corresponding R_f values and that of Ashtamangala Ghruta showed 10 peaks which are showed in Figure 2 and 3. At 366 nm the HPTLC densitometric scan showed 3 peaks in Murchita Goghruta and 7 peaks in Ashtamangala Ghruta which are showed in Figure 4 and 5. Maximum spots were observed in Ashtamangala Ghruta.

DISCUSSION

Organoleptically Murchita Go- ghruta was dark yellow in color and had characteristic ghee smell and aromatic odor of Haridra. Ashtamangala Ghruta was dark brownish

yellow in color and had a fragrant smell during the preparation with strong bitter taste. The differences in the organoleptic characters in two batches were noted. Murchita Go- ghruta and Ashtamangala Ghruta prepared in Batch-II showed organoleptically good results and hence sample of Batch-II was analyzed physico-chemically. Loss on drying indicates the moisture content in the drug. The higher value is suggestive of more amount of moisture content and the preparation is more susceptible to spoilage. In the present study, Loss on drying in Murchita Goghruta was less than Ashtamangala Ghruta. As Murchita Ghruta was subjected for processing again during the preparation of Ashtamangala Ghruta, chances of rancidity is less in Ashtamangala Ghruta. The acid value indicates the presence of free fatty acids in the sample. The free fatty acids are responsible of rancidity of the compound, flavor and the stability⁹. Higher the free fatty acids make them more rancid. Acid value was more in Murchita Ghruta

than Ashtamangala Ghruta. It suggests that Ashtamangala Ghruta contains less free fatty Acids and chances of rancidity are less when compared to Murcchita Go-ghruta. Refractive index is used in determining the identity and purity. There was no variation found in the samples and the values obtained were the same. The saponification value indicates the average molecular weight or chain length of all fatty acids present. It improves the absorption rate to the intestine there by increase nutritional value and therapeutic values. In the present study higher saponification value in Ashtamangala Ghruta shows that it contains shorter chain fatty acids so that absorption rate will be more compared to Murcchita Go-ghruta¹⁰. The iodine value indicates the degree of unsaturation of fat, which in turn denotes the less rancidity of fats and also having health benefits. In this study, Ashtamangala Ghruta contains more Iodine value which suggests the presence of higher unsaturated fatty acid bonds and the chance of rancidity will be less in it compared to Murcchita Go-ghruta. The specific gravity indicates the presence of solute content in the solvent which indicates active constituents in it. Here, specific gravity of Murcchita Go-ghruta was 0.91533 where as in Ashtamangala Ghruta it was 0.91733. It indicates that Ashtamangala Ghruta possess more active constituents compared to Murcchita Ghruta. It can be presumed that due to the process of murचना more active principle may get dissolved in the finished product leading to high therapeutic efficacy than the Murcchita Go-ghruta. If the viscosity of the liquid preparation is increased, the rate of absorption is decreased. In this study viscosity of Murcchita Ghruta was 40 minutes/ml and that of Ashtamangala Ghruta was 39 minutes/ml which indicates that absorption rate would be more in Ashtamangala Ghruta. Esters are the fatty acids with glycerol if the ester value is increased chance of rancidity is decreased. In this study, Ester value of Murcchita Ghruta was 196.465 and that of Ashtamangala Ghruta was 200.349. It suggests that chance of rancidity is less in Ashtamangala Ghruta. Unsaponifiable matter indicates the non-fatty matter which contains active components. In Murcchita Go-ghruta unsaponifiable matter was 0.6426 and in Ashtamangala Ghruta was 0.9219. Increased value in Ashtamangala Ghruta suggests that it contains more non-fatty active volatile components. HPTLC study was done just to obtain the fingerprints of the preparation and it was also done to get standard markers for the study. In the present study, HPTLC densitometric scan of chloroform extract of unsaponifiable matter of Murcchita Goghruta at 254 nm showed 8 peaks which covered the area of corresponding R_f values and that of Ashtamangala Ghruta showed 10 peaks. At 366 nm the HPTLC densitometric scan showed 3 peaks in Murcchita Go-ghruta and 7 peaks in Ashtamangala Ghruta. Maximum spots were observed in Ashtamangala Ghruta which indicates more active

constituents in it. These are the standard markers of the components and can be used as referral standards.

CONCLUSION

Murcchita Go-ghruta and Ashtamangala Ghruta were prepared as per the standard operative procedure mentioned in the classics. Both the samples were subjected to physico-chemical analysis and HPTLC. In the present study it was found that Saponification value, Iodine value, Ester value and Specific gravity were higher in Ashtamangala Ghruta than in Murcchita Go-ghruta which indicates higher active constituents were present in Ashtamangala Ghruta. The variations found in the analytical values also indicate that Murचना process can reduce the chance of rancidity thereby increase the quality of Ashtamangala Ghruta. HPTLC showed that maximum number of spots in Ashtamangala Ghruta than in Murcchita Go-ghruta at UV 254 nm and UV 366 nm. The marker compounds found in HPTLC can be identified and can be used as referral standards. Hence the present work may be used for the quality assessment and standardization of Ashtamangala Ghruta.

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