INTERNATIONAL
AYURVEDIC MEDICAL
JOURNAL

IAMJ

International Ayurvedic Medical Journal, (*ISSN: 2320 5091)* (August, 2017) 5(8)

ANALYTICAL STUDYOF AMRITABHALLATAKA GHRITA

Reddy Ravindra[1], Doddamani. M. S[2]

[1]Final year PG Scholar, [2]Guide and Head
Department of Rasashastra and Bhaishajya Kalpana, PG Studies, Taranath Government Ayurvedic Medical College and Hospital, Ballari, Karnataka, India

Email: ravindrareddy196@gmail.com

ABSTRACT

Amritabhallataka Ghrita (ABG) is a compound Ayurvedic preparation, comes under *snehakalpana*, prepared by using *Shuddha Bhallataka*, *Godugdha*, *Murchita Ghrita& sharkara*. As it contains *Bhallataka* which is mentioned as a*vishadravya in* schedule E (1) of Drug and Cosmetic Act, one cannot administer it without assessing its quality parameters. Analytical study provides us the objective parameters for standardization. It helps in understanding and interpretation of a drug and its structure. It assists us to conduct the comparative study among various samples during drug manufacturing, by offering objective parameter to judge the exact status of the drug and to decide the future work plan. Analytical study for standardization of *ABG* was carried out on the basis of classically illustrated organoleptic tests and modern parameters i.e. Physico chemical properties were carried out where Refractive index at 25^0C is 1.4609, Specific gravity of0.9881; rancidity test shows no oxidization of fat. The crucial parameters like acid value, Saponification value, Iodine value, 0.54, 219.62 and 31.93 respectively with zero Peroxide value and microbial load. Chromatological technique like High Performance Thin Layer Chromatography (HPTLC) shows 6 spots in 254nm and 5 spots in 366nm wavelength.

Keywords: *Amritabhallataka Ghrita*, Analytical study, HPTLC.

INTRODUCTION

Bhaishajya kalpana has in it a perfect blend of herbal, herbo-mineral and mineral preparations. The *Bheshaja* lies as the pride and part of Ayurveda as it is one among *chikitsa-chatushpada*. It is believed that every *dravya* available on this earth has medicinal property in it, only thing is it should be used rationally, furthermore it is said that even a poison can be a good medicament if used with caution. On

the contrary, improper use of drug will be fatal like poison, weapon, fire and thunderbolt.

Sneha kalpana proved itself as the better by completing and conquering each need of a good formulation. It incorporates the qualities of the drugs added to it without losing its own qualities. So it is sure that the fat soluble active principle of the drugs added to *sneha* can be easily extracted in it. Moreover the quality of *ghrita* is *medhya, smritikara* and it is best because of its *yogavahiguna.*

Amrita Bhallataka Ghrita is a compound formulation of ayurveda which comes under *sneha kalpana*. If we go through the classics, the yoga *Amritabhallataka Ghrita*[1]is found in various texts in various *Rogadhikara*, here the *Amritabhallataka Ghrita* explained in context of *Rasayana adhikara* was selected. It is prepared by using *Shuddha Bhallataka, Godugdha, murchita Ghrita* and *sharkara churna*. As it contains *Bhallataka* as a main ingredient which is mentioned as a *vishadravya* in Drug and Cosmetic Act hence one cannot administer it without assessing its quality parameters, so the present study aims to analyse *Amritabhallataka Ghrita* by qualitative and quantitative parameters. In classic literature, Acharyas has mentioned *gandha varna rasotpatti* for prepared *sneha* as *a siddhi lakshana* means prepared *sneha* should have *gandha, varna* and *rasa* as that of its ingredients. To interpret the same there are few modern parameters has been explained in PLIM Govt. of India Gaziyabad, among them tests like Refractive index[2], Specific gravity[3], Rancidity, Acid value[5], Saponification value[6], Iodine value[7], Peroxide value[8], HPTLC and microbial load were assessed.

AIM AND OBJECTIVE:

- Analytical standardization of *Amritabhallataka Ghrita.*
- To carryout Physico-chemical analysis of *Amritabhallataka Ghrita*.

MATERIAL AND METHODS:

The raw materials like *Sharkara, Goghrita , Godugdha* and *Gomutra* were procured from local source Ballari and *Bhallataka* was procured from *Molakalmuru* (Chitradurga dist, Karnataka).The *shodhana* of *Bhallataka* was carried out by *sthapana* method in *godugdha* for 7 days followed by *gomutra* for 7 days and further *ishtika churna gharshana*was done. *Amritabhallataka Ghrita* was prepared as per AFI reference, in pharmacy section of PG. Dept of Rasashastra & Bhaishajya kalpana, Taranath Government Ayurvedic Medical College, Ballari.

Physico chemical analysis was carried out with ancient and modern parameters.

ANALYTICAL STUDY: To assess its quality of prepared *Amritabhallataka Ghrita* was subjected to both classical and modern parameters.

A. Classical parameters

The ancient Parameters like *varna, gandha* etc. were carried out for A.B.G.in Rasashastra and Bhaishajya Kalpana Dept. TGAMC, Ballari, details are mentioned in table 1

Table 1: Showing Organoleptic characters of A.B.G:

Parameters	*ABG*
Varna	*Peeta*
Rasa	*Katu*
Gandha	Characteristic
Sparsha	*Snigdha*
Shabda	Nil

Physico-Chemical Tests:

Physical tests like organoleptic, acid value, refractive index, specific gravity, rancidity, saponification value, iodine value, peroxide value and biomarker HPTLC were conducted in SDM Research Centre laboratory for Ayurveda and Allied Sciences, Udupi.

Modern parameters:

B. Physical tests

1. Organoleptic characters

Colour, odour, taste of the given sample was tested using sensory organs, and the same were noted in table no 2.

Refractive index[2]:

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre. Noted the reading. Distilled water has a refractive index of 1.3325 at 25˚C. The difference between the reading and1.3325 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 28˚C.

Specific gravity[3]:

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room Temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

Rancidity[4]:

Mixed 1.0ml of melted fat and 1.0ml of conc. Hcl in a test tube, add 1.0ml of 1% Phloroglucinol in diethyl ether and mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized, while a red color indicates that the fat is definitely oxidized.

Acid value[5]:

Weighed 2- 10g of ghee in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 +25ml) previously neutralised with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink colour. Repeated the experiment twice to get concordant values.

Saponification value[6]:

Weighed 2g of the Oil / Fat into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash.Refluxed on a water bath for 30 minutes. Cooled and added 1 ml of Phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). Repeated the operation omitting the substance being examined (blank) (b ml). Repeated the experiment twice to get concordant values.

Iodine value[7]:

The sample was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl_4, 20ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17^0 C for 30 min. 15ml of potassium iodide and 100ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

Peroxide value[8]:

5g of the *Ghrita* was weighed accurately into a conical flask, added 30 ml of mixture of 3volumes of glacial acetic acid and 2 volumes of chloroform, added 0.5ml of potassium iodide, allowed it to stand for 1 minute, add 30ml of water titrate gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow colour disappears. Add 0.5ml of starch indicator continued the titration until blue colour disappears.

Peroxide value= 10(a-b)/W

Where W= weight in g of the substance.

High Performance Thin Layer Chromatography[9]:

3,6 and 9µl of the sample was applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator.

For *Amritabhallataka Ghrita* the plate was developed in Toluene: Ethyl Acetate (9.0: 1.0), the developed plates were visualized in UV 254nm, 366nm and then derivatives with an aldehydesulphuric acid reagent and scanned under UV 254nm, 366nm and 620nm post derivatisation. R_f, colour of the spots and densitometry scan were recorded.

Solvent system – Toluene: Ethyl Acetate (9.0: 1.0)

Total microbial count[10]:

Preparation of Casein Soya bean Digest Agar Medium (CSDAM):

Dissolve casein peptone (15 g), soya peptone (5 g), Sodium Chloride (5 g) were taken and dissolved in 990 ml distilled water and pH was adjusted to 7.3±0.2 and make up the volume to 1000 ml. Finally add 15 g of agar to the media and autoclaved at 121˚C for 20 minutes.

Preparation of Buffered Sodium chloride Peptone Solution (BSCPS) pH 7.0:

Dissolve potassium dehydrogenate phosphate (3.56 g), disodium hydrogen phosphate (7.23 g), Sodium Chloride (4.3 g), peptone (1.0 g) were taken and dissolved in 990 ml distilled

water. The pH was adjusted to 7.0 and make up the volume to 1000 ml. Then above buffer solution was autoclaved at 121˚C for 20 minutes.

There is no growth of microbes in the sample *Amritabhallataka Ghrita*, detail is tabulated in table no-5.

RESULTS:

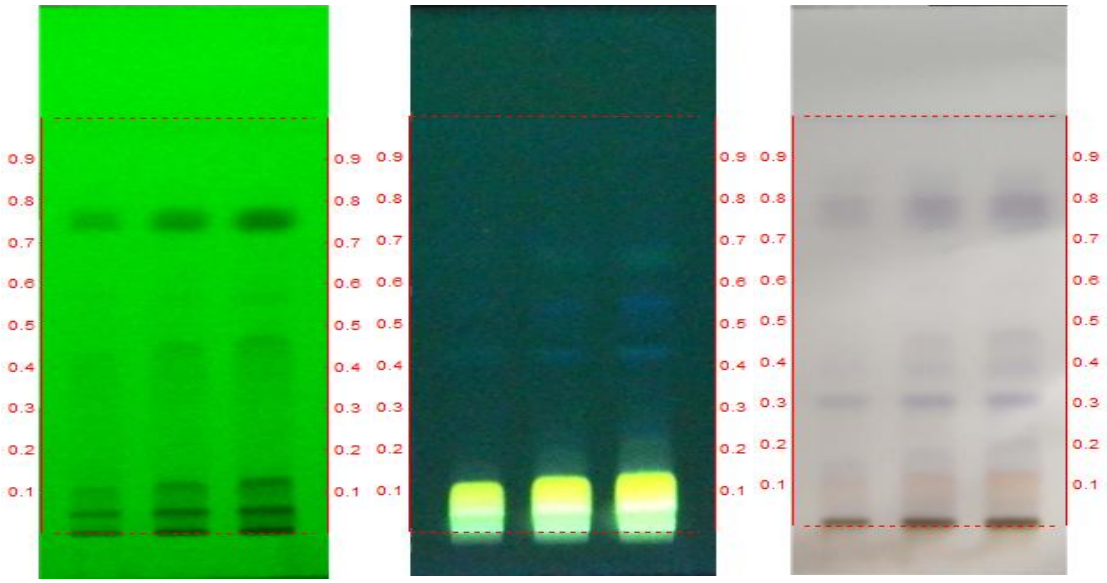
Table 2: Showing Organoleptic characters of A.B.G:

Physical test	A.B.G
Colour	Yellow
Odour	Characteristic
Taste	Acrid

Table 3: Showing Results of standardization parameters

Parameter	*Results n = 3 %w/w*
	AB Ghrita
Refractive index	1.4609
Specific gravity	0.9881
Rancidity	Fat is not oxidized
Acid value	0.54
Saponification value	219.62
Iodine value	31.93
Peroxide value	0.00

Figure 1: HPTLC photo documentation of ethanol extract of AB Ghrita



Short UV **Long UV** **Post derivatisation**

Track 1-AB Ghrita– 3µl
Track 2-AB Ghrita – 6µl
Track 2-AB Ghrita – 9µl

Table 4: Showing R_f values of samples

Short UV	Long UV	Post derivatisation
0.06 (D. green)	-	0.06 (Purple)
-	0.10 (Yellow)	-
0.13 (D. green)	-	0.13 (Pink)
-	0.19 (L. green)	0.19 (Purple)
-	-	0.31 (Purple)
-	-	0.40 (Purple)
-	0.43 (F. blue)	-
0.45 (D. green)	-	0.45 (Purple)
-	0.56 (F. blue)	-
0.58 (D. green)	-	-
0.63 (L. green)	-	-
-	0.67 (F. blue)	-
0.76 (D. green)	-	-
-	-	0.80 (Purple)
-	-	0.86 (Purple)

*F – Fluorescent; L –Light; D – Dark

Figure 2: Densitometric scan at 254nm

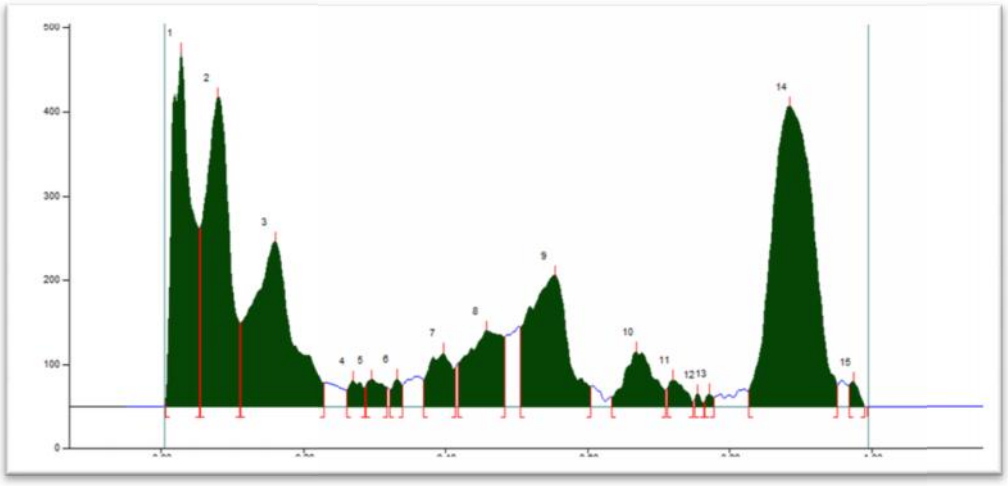


Fig 2a. AB Ghrita – 9µl

Track 8, ID: A.B ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.7 AU	0.03 Rf	420.0 AU	22.03 %	0.05 Rf	10.4 AU	8172.6 AU	14.97 %
2	0.06 Rf	212.9 AU	0.08 Rf	367.0 AU	19.25 %	0.11 Rf	99.7 AU	8748.9 AU	16.03 %
3	0.11 Rf	99.8 AU	0.16 Rf	196.1 AU	10.28 %	0.23 Rf	28.1 AU	7839.2 AU	14.36 %
4	0.26 Rf	19.8 AU	0.27 Rf	31.1 AU	1.63 %	0.29 Rf	22.3 AU	442.2 AU	0.81 %
5	0.29 Rf	24.3 AU	0.30 Rf	32.2 AU	1.69 %	0.32 Rf	23.3 AU	531.8 AU	0.97 %
6	0.32 Rf	20.0 AU	0.33 Rf	33.1 AU	1.74 %	0.34 Rf	26.1 AU	329.9 AU	0.60 %
7	0.37 Rf	31.8 AU	0.40 Rf	63.9 AU	3.35 %	0.41 Rf	46.7 AU	1496.8 AU	2.74 %
8	0.42 Rf	51.9 AU	0.46 Rf	90.9 AU	4.77 %	0.48 Rf	83.5 AU	3115.3 AU	5.71 %
9	0.51 Rf	94.7 AU	0.55 Rf	156.7 AU	8.22 %	0.60 Rf	23.8 AU	6037.5 AU	11.06 %
10	0.64 Rf	11.4 AU	0.67 Rf	65.2 AU	3.42 %	0.71 Rf	19.8 AU	1884.2 AU	3.45 %
11	0.71 Rf	20.7 AU	0.72 Rf	31.7 AU	1.66 %	0.75 Rf	6.0 AU	535.7 AU	0.98 %
12	0.75 Rf	8.0 AU	0.76 Rf	15.0 AU	0.79 %	0.76 Rf	4.8 AU	90.8 AU	0.17 %
13	0.77 Rf	6.4 AU	0.77 Rf	15.7 AU	0.82 %	0.78 Rf	10.8 AU	107.3 AU	0.20 %
14	0.83 Rf	18.6 AU	0.89 Rf	357.4 AU	18.75 %	0.95 Rf	25.9 AU	14966.4 AU	27.42 %
15	0.97 Rf	25.4 AU	0.98 Rf	30.3 AU	1.59 %	0.99 Rf	0.6 AU	283.8 AU	0.52 %

Figure 3: Densitometric scan at 366nm

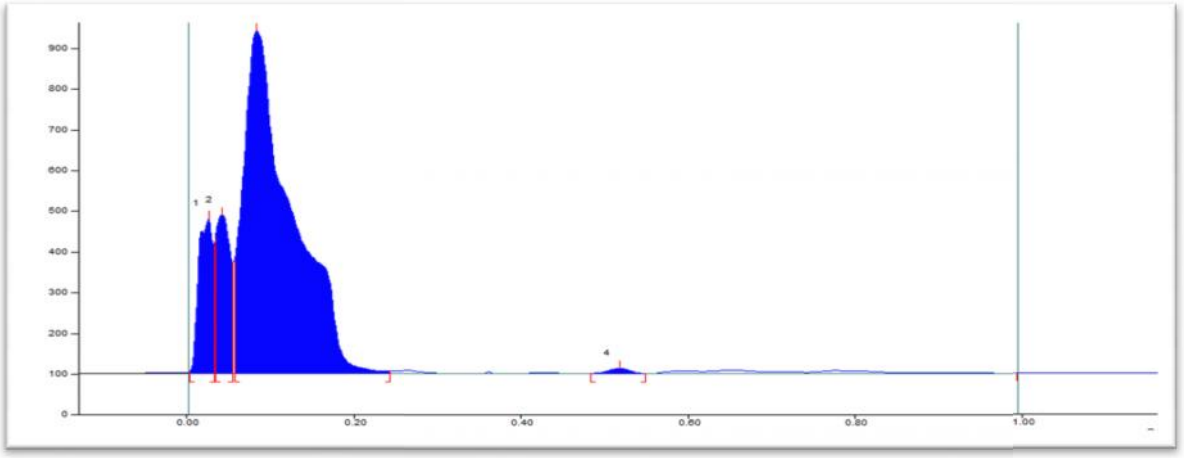


Fig 3a. AB Ghrita – 9µl

Track 8, ID: A.B ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	4.4 AU	0.03 Rf	379.5 AU	23.39 %	0.03 Rf	14.2 AU	4781.0 AU	10.69 %
2	0.04 Rf	333.3 AU	0.04 Rf	388.7 AU	23.96 %	0.06 Rf	71.0 AU	4836.9 AU	10.82 %
3	0.06 Rf	278.4 AU	0.08 Rf	841.5 AU	51.87 %	0.24 Rf	5.2 AU	34866.6 AU	77.96 %
4	0.48 Rf	0.7 AU	0.52 Rf	12.6 AU	0.78 %	0.55 Rf	0.1 AU	236.3 AU	0.53 %

Figure 4: Densitometric scan at 620nm

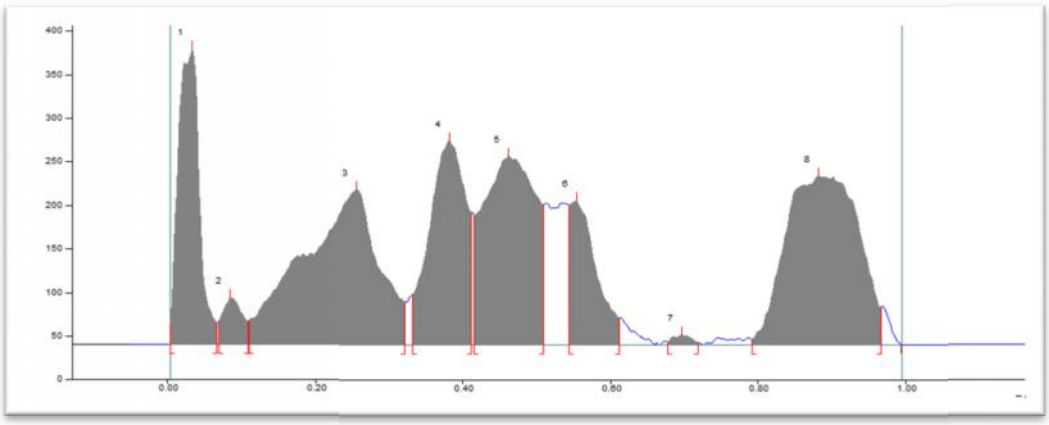


Fig 4a: AB Ghrita – 9µl

Track 8, ID: A.B ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	23.0 AU	0.03 Rf	338.7 AU	24.38 %	0.07 Rf	25.0 AU	6962.6 AU	11.86 %
2	0.07 Rf	25.5 AU	0.09 Rf	53.2 AU	3.83 %	0.11 Rf	26.9 AU	1043.6 AU	1.78 %
3	0.11 Rf	27.2 AU	0.26 Rf	178.1 AU	12.82 %	0.32 Rf	49.3 AU	13021.3 AU	22.18 %
4	0.33 Rf	57.9 AU	0.38 Rf	233.2 AU	16.79 %	0.41 Rf	51.7 AU	8288.0 AU	14.11 %
5	0.42 Rf	149.2 AU	0.46 Rf	215.6 AU	15.53 %	0.51 Rf	60.8 AU	10968.3 AU	18.68 %
6	0.55 Rf	160.6 AU	0.55 Rf	165.5 AU	11.92 %	0.61 Rf	30.8 AU	4277.5 AU	7.28 %
7	0.68 Rf	3.1 AU	0.70 Rf	11.2 AU	0.81 %	0.72 Rf	3.0 AU	206.5 AU	0.35 %
8	0.79 Rf	5.9 AU	0.88 Rf	193.4 AU	13.92 %	0.97 Rf	42.6 AU	13951.3 AU	23.76 %

Table 5: Showing Microbial load analysis of *Amritabhallataka Ghrita*

Sl. No.	Dilutions	Number of Colonies (NOC)		CFU/ml
1	Direct	0	0	0
2	1/10 (10^1)	0	0	0

CFU - Colony Forming Units

DISCUSSION

Physico-chemical parameters

Refractive index- It is the ratio of the velocity of light in a vacuum to its velocity in the substance. It is a fundamental physical property of a substance often used to identify a particular substance, confirm its purity, or measure its concentration. More will be Refractive Index, there will be more concentration of light which facilitates rancidification of Ghrita i.e., decomposition of *Ghrita*.

Refractive index of the *ABG* was 1.4609.

Specific gravity– It indicates the solid to liquid ratio in the *Ghrita*. Specific gravity of the *ABG* was 0.9881.

Rancidity– Ghee and fat with higher degree of unsaturation will pick up oxidative rancidity earlier. Volatile products which are produced by complex chemical changes due to high peroxide level are responsible for rancid taste and odour.

Fat is not oxidized in *Amritabhallataka Ghrita*.

Acid value–It is a measure of the amount of Carboxylic acid groups in a chemical compound, such as fatty acid, or in a mixture of compounds as oil-fats rancidify, triglycerides are converted into fatty acids and glycerol, causing an increase in acid. Less acid value denotes the less chance of decomposition of Ghrita thus increasing both life span and therapeutic value.

The acid value of the *ABG* was 0.54.

Saponification value –Saponification value gives an idea about the molecular weight of an oil / Fat. The saponification value and molecular weight of oil are inversely proportion. It is helpful in determining adulteration of given fat by one of the lower or higher saponification value.

Saponification value of the *ABG* is 219.62.

Iodine value- It indicates the degree of unsaturation. Greater degree of unsaturation indicates the possibility of the ghee becoming rancid due to atmospheric oxidation. And the iodine value of the *ABG* was 31.93.

Peroxide value- it is the most widely used analytical method. It gives a measure of the extent to which an oil/ghee sample as undergone primnaryoxidation, extent of secondary oxidation may be determined from p-anisidine test. Peroxide value of the *ABG* was zero.

Discussion on HPTLC

HPTLC is the sophisticated analytical parameter for the evaluation of the herbal drugs. HPTLC can also serves as Fingerprinting technique for identification and quantification of the herbal and herbo-mineral formulations. Through HPTLC technique major phytochemical present the drug or formulation can be estimated. It helps to find out the adulteration in the formulation and is used as a standard for the herbal compounds.

Discussion on Microbial limit test.

1. Total Bacterial Count: Total microbial count in the sample *ABG* is free from Microbial contamination, showing that the collected raw material was genuine in processing the formulation.
2. Total Fungal Count: Total fungal count in the sample *ABG* is free from fungal contamination; the collected raw material was genuine in processing the formulation.

CONCLUSION

Physical test shows *ABG* is light yellow in colour with acrid taste and characteristic odour. Quantitative chemical analysis shows that in *ABG*, refractive index-1.4609, Specific gravity-0.9881, Rancidity-fat is not oxidized, Acid value-0.54, Saponification value-219.62, Iodine value-31.93 and peroxide value- zero. HPTLC study of *ABG* and other herbal ingredients confirms the presence of Rf value and colour bands of Shuddha Bhallataka in *ABG*. In HPTLC study presence of bands of *Bhal-*

lataka shows importance of *snehapaka*, where all the contents of *Bhallataka* transferred to *ABG*.

ACKNOWLEDGEMENT

I thank principal and teaching staff of post graduate studies in Rasashastra and Bhaishajya Kalpana of TGAMC Ballari for their support and encouragement.

REFERENCES

1. Anonymous-The Ayurvedic Formulary of India, second revised English edition, Govt. Of INDIA Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homeopathy, NEW DELHI: Published by The Controller of Publications Civil Lines Delhi; 2003.Part-1, 6thSection , 80pp.
2. Anonymous. The Ayurvedic pharmacopoeia of India, Government of India Ministry of health and family welfare, Department AYUSH,New Delhi:2004. the controller of publications civil lines., part-1, Volume-2, 190pp.
3. Frank T. Welcher, Standard Method of Chemical Analysis, New Delhi:Industrial and Natural Products and Non Instrumental Methods and Robert K. Krieger Publishing company; Vol.II Part B, Chapter.-33.
4. Anonymous, Oils and Fats Dept. of Chemistry, Saurashtra University.
5. Anonymous. The Ayurvedic pharmacopoeia of India, Government of India, Ministry of health and family welfare, Department AYUSH, New Delhi: 2004.the controller of publications civil lines., part-1, Volume-2, 201pp.
6. Anonymous. The Ayurvedic pharmacopoeia of India, Government of India, Ministry of health and family welfare, Department AYUSH, New Delhi:2004. the controller of publications civil lines., part-1, Volume-2, 199pp.
7. Anonymous. The Ayurvedic pharmacopoeia of India, Government of India,Ministry of health and family welfare, Department AYUSH, New Delhi:2004. the controller of publications civil lines., part-1, Volume-2, 200pp.
8. Anonymous. The Ayurvedic pharmacopoeia of India, Government of India,Ministry of health and family welfare, Department AYUSH, New Delhi:2004. the controller of publications civil lines., part-1, Volume-2, 201pp.
9. Kasture A.V. Mehadik R. et.al. Pharmacopieal Analysis. Pune: NiraliPrakashan; 2002.vol 5.13-18pp.
10. Anonymous. The Ayurvedic pharmacopoeia of India, Government of India,Ministry of health and family welfare, Department AYUSH, New Delhi:2004. the controller of publications civil lines., part-1, Volume-2, 163pp.

Source of Support: Nil
Conflict Of Interest: None Declared

How to cite this URL: Reddy Ravindra & Doddamani. M. S:Analytical Studyof Amritabhallataka Ghrita. International Ayurvedic Medical Journal {online} 2017 {cited August, 2017} Available from: http://www.iamj.in/posts/images/upload/2812_2821.pdf