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PHARMACOGNOSTICAL AND PHYTOCHEMICAL SCREENING OF *SAUBHAGYANADANA GHRITA*

Jalpa Deepak Rupareliya [1] Shilpa B. Donga [2] C R Harisha[3] Abhay Jayprakash Gandhi [4]

1. M.S. Scholar, Department of Prasuti Tantra and Streeroga,
2. Professor, Department of Prasuti Tantra and Streeroga,.
3. Head, Pharmacognosy
4. Ph.D. Scholar, Pharmaceutical Chemistry, IPGT & RA, Gujarat Ayurved University, Jamnagar.

Address for correspondence:

Jalpa Deepak Rupareliya, MS Scholar, Department of Prasuti Tantra and Streeroga, IPGT & RA, Gujarat Ayurved University, Jamnagar.
Email ID: - Jalparupareliya20@gmail.com

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ABSTRACT

Background

Saubhagyanadana Ghrita is referred in *Abhinava Navjeevana* text narrated by Siddhinandana Mishra in *Vandhya Chikitsa Adhyaya*. It is indicated in almost all types of *Vandhya Roga*. It also states that usage of this *Ghrita* doesn't only cures infertility but also provides a healthy progeny to the lady. *Saubhagyanadana Ghrita* is an unexplored formulation and so has to be studied in details.

Aim

The present study was aimed to set a manufacturing protocol, and to develop pharmacognostical and pharmaceutical profile of *Saubhagyanandan Ghrita.*

Material and Method

Study included preparation of *Saubhagyanandan Ghrita* following all SOPs using raw drugs, which were previously authenticated. Later, *Saubhagyanandan Ghrita* was subjected to pharmacognostical, qualitative parameters and high performance thin-layer chromatography (HPTLC) analysis as per standard protocols.

Result and Discussion

The pharmacognostical study reveals the presence of characteristics features of all the drugs present in the composition. Pharmaceutical analysis showed that specific gravity at 40^0C is 0.9181, Refractive index at 40^0C is 1.48, Acid value is 3.352, Saponification value 14.06%, Ester value is 10.708, unsaponifiable matter is 3.88% w/w, iodine value is 81.54g, rancidity test is negative. HPTLC fingerprinting profile of *Saubhagyanadana Ghrita* revealed 10 spots at 254 nm and 08 spots at 366nm.

Conclusion

The present investigation will be helpful in assessing the pharmacognostical, phytochemical analysis and laying down pharmacopoeial standards for *Saubhagyanadana Ghrita.*

Keyword: - HPTLC, Pharmacognosy, Phytochemical, *Saubhagyanadana Ghrita.*

INTRODUCTION

Every creature in this universe tries to keep its progeny. *Putra Eshana* is the strongest desire of all the married couple. Infertility is defined as an inability to conceive a pregnancy after one year of unprotected intercourse. [1] It can either be primary where no previous pregnancy has occurred or secondary where there has been a previous documented pregnancy. *Vandhyatva*(Infertility) is recognized since Vedic period. All types of female infertility in Ayurveda are described under the heading of *Vandhya*. *Acharya* Sushruta included *Vandhya* in *Yonivyapada* and in *Artav-vaha Strotas Viddha Lakshanas*.[2]

Saubhagyanadana Ghrita[3] is referred in *Abhinava Navjeevana* text narrated by Siddhinandana Mishra in *Vandhya Chikitsa Adhyaya.* It has properties like *Snehana, Bruhana, Balya, Rasayana* and *Garbha Sthapaka.* It is indicated in almost all types of *Vandhya Roga.* It also states that usage of this *Ghrita* doesn't only cures Infertility but also provides a healthy progeny to the lady. Though many work has been carried out in infertility using various drugs through oral, anal and uterine route. *Saubhagyanandana Ghita* is unexplored yet. Its indications are wide and seems to be fruitful so here a work was carried out for development of its manufacturing protocol and assessing the pharmacognostical, and phytochemical analysis. Most of the drugs in this composition is of *Madhura, Tikta Rasa* and having *Vata-Pitta-ghna* properties. Further properties are as shown in Table 1.

Table 1: Pharmacokinetic action of the ingredients of *Saubhagyanandana Ghruta.*[4]

Name of drug	Latin Name	Part used	Ratio	Rasa	Guna	Virya
Kalka Dravya						
Shatavari	*Asparagus racemosa* Willd.	Root	1 part	*Tikta Madhura*	*Guru, Snigdha,*	*Sheeta*

Ashwagandha	*Withania somnifera* Linn.	Root	1 part	*Tikta Kashaya*	*Guru, Snigdha,*	*Ushna*
Aparmarga	*Achyranthes aspera* Linn.	Root	1 part	*Tikta, Katu*	*Sara Teekshna*	*Ushna*
Bala	*Sida cordifolia* Linn.	Root	1 part	*Madhura*	*Snigdha*	*Sheeta*
Matulunga	*Citrus medica* Linn.	Root	1 part	*Madhura Amla*	*Laghu Snigdha,*	*Ushna*
Eranda	*Ricinus communis* Linn.	Root	1 part	Madhura	*Guru, Snigdha,*	*Ushna*
Nagakesara	*Mesua ferrea* Linn.	Stamens	1 part	*Tikta Kashaya*	*Laghu Ruksha*	*Ushna*
Aatmagupta	*Mucuna pruriens* Bek.	Root	1 part	*Madhura Tikta*	*Guru Snigdha*	*Ushna*
Drava Dravya						
Vatashrunga	*Ficus bengalensis* L	Leaf bud	64 parts	*Kashaya Madhura*	*Ruksha Guru*	*Sheeta*
Godugdha	Animal product	-	64 parts	*Madhura*	*Guru, Snigdha,*	*Sheeta*
Goghrita	Animal product	-	16 parts	*Madhura*	*Guru, Snigdha,*	*Sheeta*
Prakshepa Dravya						
Nagakesara	*Mesua ferrea* Linn.	Stamens	1 part	*Tikta Kashaya*	*Laghu Ruksha*	*Ushna*

MATERIAL AND METHODS

Collection and Authentication of Raw Drugs [5]

Ashwagandha, Apamarga, Baalmoola, Erandamoola, Nagakeshara was collected from the Pharmacy, Gujarat Ayurved University, Jamnagar. *Shatavari, Matulunga, Aatmagupta,, Vatashrunga, Godugdha and Goghrita* was collected from the local market of Jamnagar. Identification and authentication of all procured drugs was done from Pharmacology laboratory of IPGT & RA Jamnagar before manufacturing.

Method of preparation of *Saubhagyanandan Ghrita*

The whole ghrita was completed in 3 siddhis. Freshly collected *Vatashrunga* was soaked in water overnight and *Kwatha* prepared by boiling it to 1/8th. All *Kalka Dravyas* was taken in *Churna* form and a bolus was formed by adding sufficient amount of water. *Goghrita* was taken and heated to melt it. Then *Kalka Dravyas* was added followed by *Vatshrunga Kwatha*. One *Siddhi* was completed after appearance of all *Siddhi Lakshanas*. In 2nd *Siddhi,* same procedure was repeated by using *Godugdha* as *Drava Dravya*. In 3rd *Siddhi*, same procedure was repeated by using water as *Drava Dravya*.

Pharmacognostical Analysis

Pharmacognostical analysis of *Saubhagyanandana Ghrita* based on organoleptic characters, i.e. colour, odour, taste and texture were recorded. Microscopic studies of the raw drugs with and without stain to find out the lignified materials along with other cellular constituents was done. The micro photographs were taken under Carl Zeiss Trinocular microscope attached with camera. [7]

Pharmaceutical Analysis

Physicochemical study of sample was carried out by using various physicochemical parameters as mentioned in Ayurvedic Pharmacopeia of India, 2001. *Saubhagyanandana* Ghrita used as a sample. [8] Following parameters were performed.

Determination of Specific gravity at 40°C.

A pycnometer of 25 ml, capacity is cleaned, dried and weighed. It is filled up to the mark with water at the required temperature and weight. The pycnometer is next filled up to the mark with the sample, at the same temperature and weighed; the specific gravity is determined by dividing the weight of the sample in grams by the weight of the water, expressed in grams.

Determination of Refractive index at 40°C.

Attach the prism box of Abbe's refractometer to a thermostatic bath regulated at 40°C. Open the prism box and place a few drops of sample on the lower prism, close the box. Adjust the mirror to give a bright illumination of the field. Turn the Krunled knob until the field has a light and dark section. If there is a coloured fringe between the two areas, adjust the Amici prisms until the boundary is sharp and black, set it on the cross hairs, and note the reading of refractive index. Open the prism box and wipe off the sample with cotton wool moistened with acetone.

Determination of Acid value.

Mix 25ml ether with 25ml alcohol (95%) and 1ml of 1% phenolphthalein solution and neutralize with N/10 alkali (few drops). Dissolve about 5gm of fat or oil accurately weighed, in the mixed neutral solvent and titrated with N/10 potassium (or sodium) hydroxide shaking constantly until a pink colour persists for 15 seconds is obtained.

Determination of Saponification value.

Weigh 2gm of the oil or fat into a conical flask and add exactly 25ml of the alcoholic potassium hydroxide solution. Attach a reflex condenser and heat the flask in boiling water for one hour, shaking frequently. Add 1ml of phenolphthalein (1%) solution and titrate the excess alkali with N/2 hydrochloric acid. Carry out a blank at the same time.

Determination of Ester value.

The ester value is the number of milligrams of potassium hydroxide required to saponify the esters present in 1gm of the substance.

Ester value = Saponification value – Acid value.

Determination of Iodine value

Iodine monochloride method (Wij's method): Place an accurately weighed quantity of the substance being examined in a dry iodine flask of 500 ml capacity, add 10 ml of CCl4 and dissolve. Add 20 ml of iodine monochloride solution. Insert the stopper and allow to stand in the dark at a temperature between 15-25 □C for 30 min place 15 ml KI solution in the cup top, carefully remove the stopper, rinse the stopper and sides of the flask with 100 ml of water, shake and titrate with 0.1 M sodium thiosulphate using starch solution added towards the end of the titration as indicator. Note the number of ml required (a). Repeat the operation omitting the substance being examined and note the number of ml required (b). Calculate the iodine value from the expression.

Determination of Unsaponifiable matter

About 5gm of the sample to be examined accurately weighed into a 250ml flask fitted with a reflex condenser. Add a solution of 2gm of potassium hydroxide in 40ml of alcohol and heat on a water bath for one hour, shaking frequently. Transfer the contents of the flask to a separating funnel with the aid of 100ml of hot water and while the liquid is still slightly warm, shake very carefully with three successive quantities, each of 100ml of solvent ether. Combine the ether extracts in a second separating funnel containing 40ml of water, swirl gently for a few minutes. Allow to separate and reject the lower layer. Wash the ether extract with two quantities, each 40ml of a 3% w/v solution of potassium hydroxide. Each treatment being followed by a washing with 40 ml of water. Finally wash the ether layer with successive quantities each of 40 ml of water until the aqueous layer is no longer alkaline to phenolphthalein. Transfer the ether layer to a weighed flask. Washing out the separating funnel with solvent ether. Distill off the ether and add to the residue 6ml of acetone. Remove the solvent completely from the flask with the aid of a gentle current of air. Dry at 100-105°C for 30 minutes, cool in desiccator and weigh the residue. Calculate the unsaponifiable matter as a percentage (w/w).

Kries test for Rancidity

Red colour is produced when 0.1% phloroglucinol in ether is allowed to react with oxidized fat in acid solution. 10ml of oil+10ml phloroglucinol + 10ml conc. HCl. shake for 20 seconds. Pink colour indicates rancidity.

HPTLC Study [7]

Instrumentation:

A CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC auto sampler 4, twin trough plate development chamber, TLC Scanner 3, win CATS software version 1.4.4. and Hamilton (Reno, Nevada, USA) Syringe.

HPTLC method:

5µl of extract was loaded on E. Merck aluminum plate pre coated with silica gel 60 F_{254} of 0.2 mm thickness and the plate was developed in Toluene: Ethyl acetate (9:1) in twin trough chamber previously saturated with solvent system. After development densitometric scan was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 and 366 nm under control of Win CATS Software (V 1.2.1. Camag) (Stahl, 1969). The plate was then dipped in sulphuric acid reagent and heated in a hot air oven at 105ºC until the colour of the spots appeared and profile photo was documented under white light.

Results and Discussion:

Organoleptic characters: The results are shown in Table 2

Table 2 : Organoleptic characters of *Saubhagyanadana Ghrita.*

Drug name	Organoleptic characteristic			
	Colour	Odour	Taste	Touch
Saubhagyanandan Ghrita	Reddish brown	Characteristics	Bitter	Contour

Microscopic characters

Powder microscopy of raw drugs of *Saubhagyanadana Ghrita* showed the striking characters of all individual drugs. The data was shown in Figure 1, Figure 2, Figure 3.

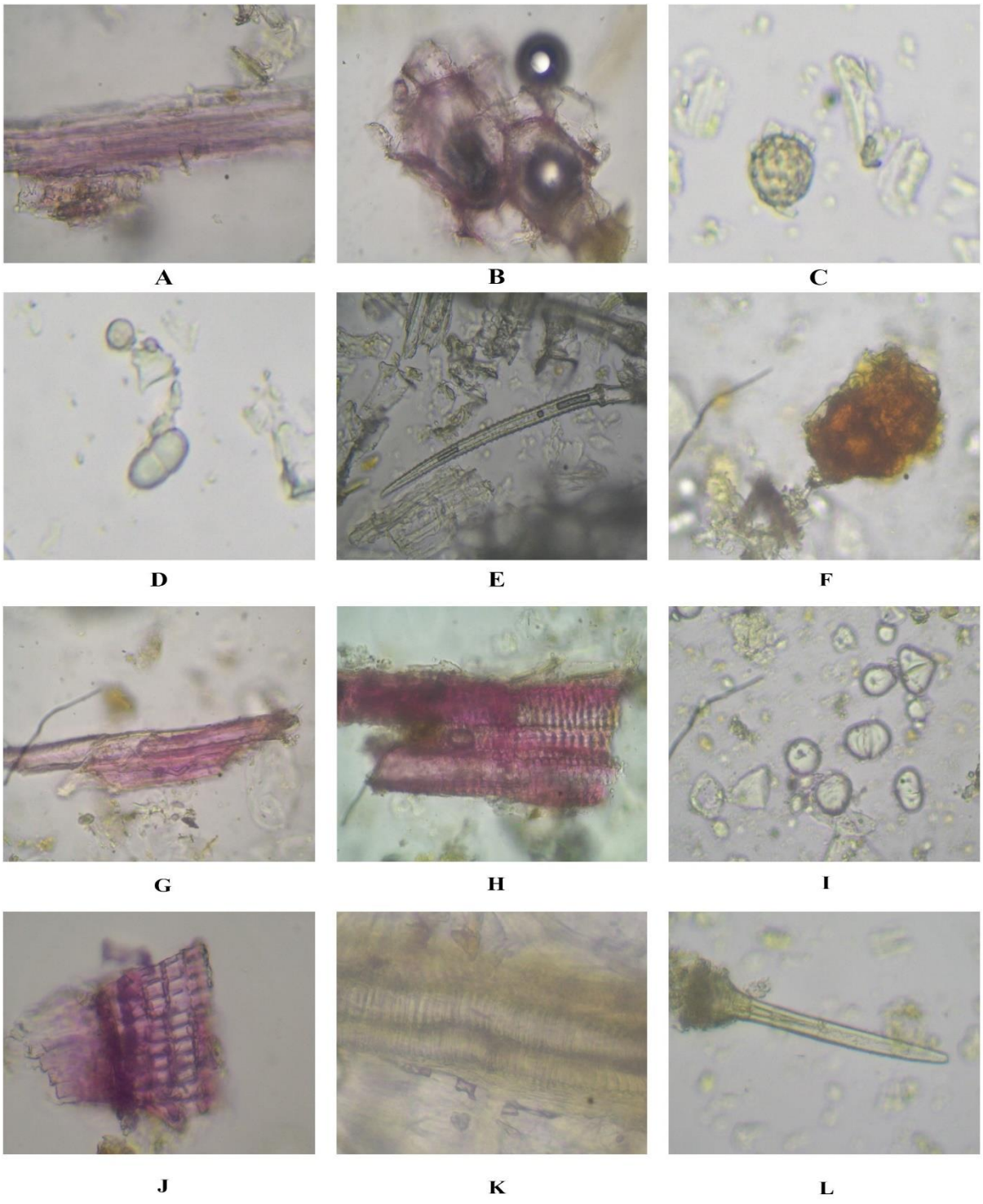

Figure 1: Pharmacognostical profile of *Saubhagyanadana Ghrita*

Where, A Lignified fibre of *Apamarga*, **B** Lignified parenchyma of *Apamarga*, **C** Pollen grain, **D** Simple and compound starch grains of *Apamarga*, **E** Simple trichome of *Apamarga*, **F** Brown constant of *Ashwagandha*, **G.** Lignified fibres of Ahwagandha, **H** Pitted vessels of *Ashwagandhai,* **I** Simple and starch grains of *Ashwagandha,* **J.** Annular vessels of *Bala,* **K** Simple trichome of *Bala*, **L** Spiral vessels of *Bala*

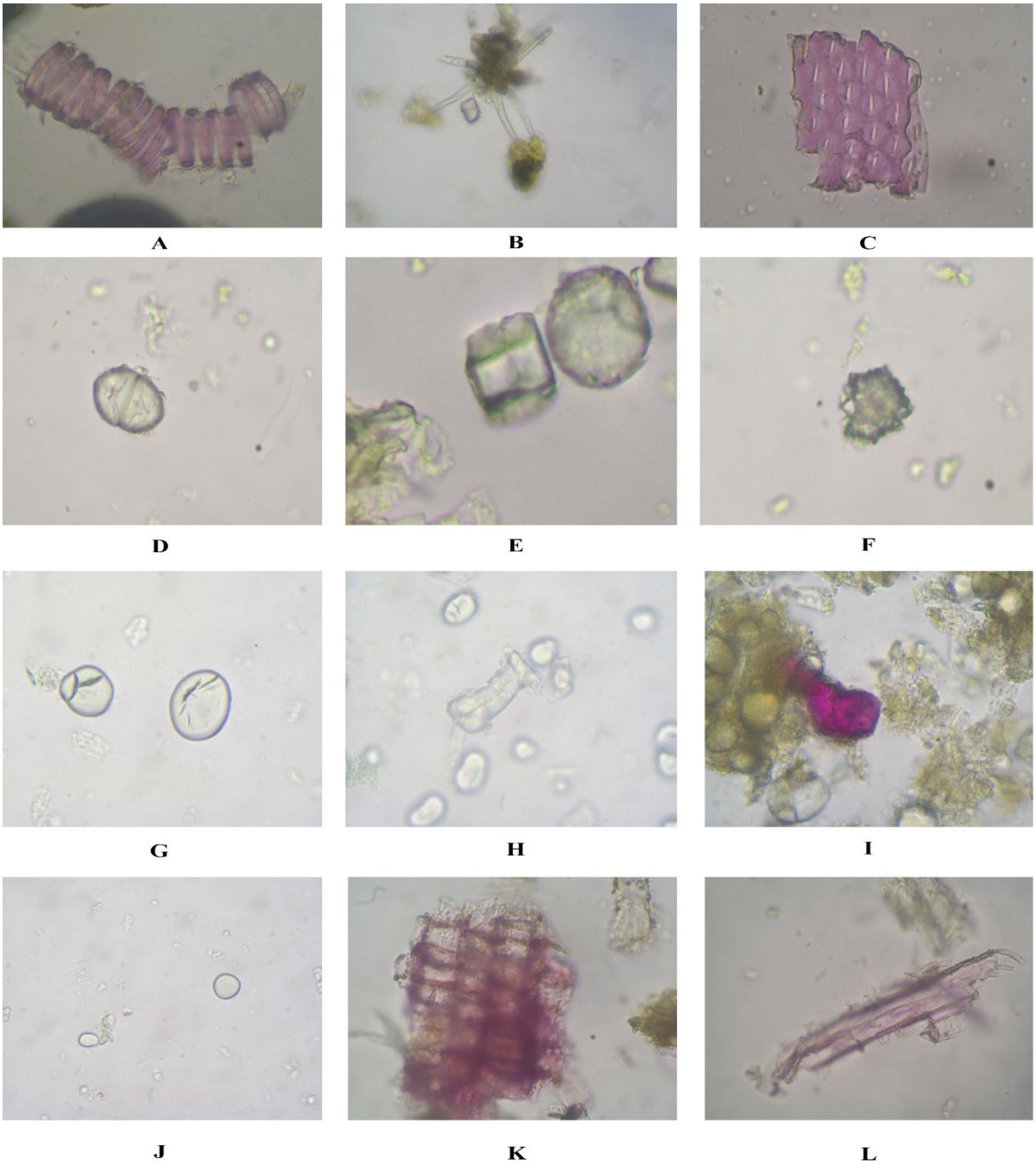

Figure 2: Pharmacognostical profile of *Saubhagyanadana Ghrita*

Where, A represents spiral vessels of *Bala*, **B** represents stellate trichome of *Bala*, **C** represents border pitted vessels of *Erandamoola*, **D** represents compound starch grains of *Erandamoola*, **E** represents Rhomboidal crystals of *Erandamoola*, **F** represents rosette crystals of *Erandamoola*, **G** represents simple starch grains with hilum of *Kapikacchu*, **H** represents spool cells of *Kapikacchu*, **I** represents stone cells of *Kapikacchu* , **J** *Simple grains of Kapikacchu* , **K** Lignified cork of *Erandamoola,* **L** lignified fibre of *Erandamoola*

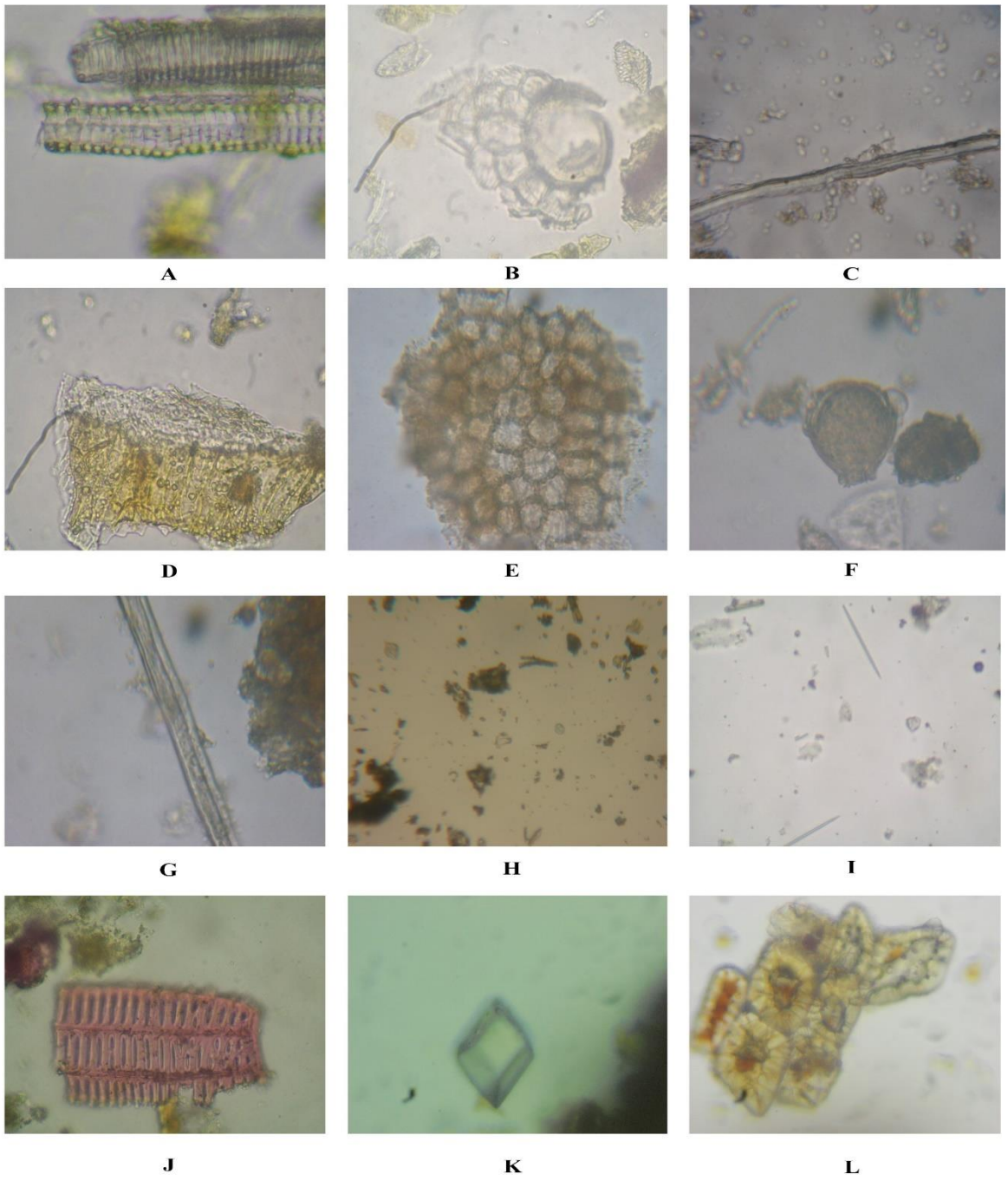

Figure 3: Pharmacognostical profile of *Saubhagyanadana Ghrita*

Where, A represents annular vessels of *Matulunga*, **B** represents endosperm of *Matulunga*, **C** represents fibre of *Matulunga*, **D** represents stratified cells with oil of *Matulunga*, **E** represents Beaded parenchyma of *Nagakesara*, **F** represents pollen grain of *Nagakesara*, **G** represents simple fibre with lumen of *Nagakesara*, **H** represents starch grains of *Nagakesara*, **I** represents acicular crystals of *Shatavari*, **J** represents Annular vessels of *Shatavari*, **K** represents prismatic crystals of *Vatashrunga*, **L** represents Stone cells with brown contrast of *Vatashrunga.*

Pharmaceutical analysis

The results of pharmaceutical anaylsis of *Saubhagyanadana Ghrita* is as shown in Table 3.

Table 3: Pharmaceutical analysis of *Saubhagyanadana Ghrita.*

Sr No.	Parameters	Values
1.	Specific gravity at 40℃	0.9181
2.	Refractive index at 40℃	1.48
3.	Acid value	3.352
4.	Saponification value	14.06%
5.	Ester value	10.708
6.	Unsaponifiable matter (% w/w)	3.88%%
7.	Iodine value	81.54 g
8.	Rancidity test	Negative

HPTLC

On performing HPTLC, the chromatogram showed 10 peaks at 254 nm; while the chromatogram showed 08 spots at 366 nm. The data was shown in Table 4. The HPTLC profile of Saubhagyanadana Ghrita was as shown in the Figure 4.

Table 4: HPTLC Profile of *Saubhagyanadana Ghrita*

Conditions	**No of Peak**	**Rf values *Saubhagyanadana Ghrita***
Short ultra violet (254 nm)	10	0.06,0.12,0.20,0.32,0.45,0.51,0.56,0.65,0.76,0.80
Long ultra violet (366 nm)	08	0.06,0.12,0.20,0.32,0.41,0.51,0.65,0.76

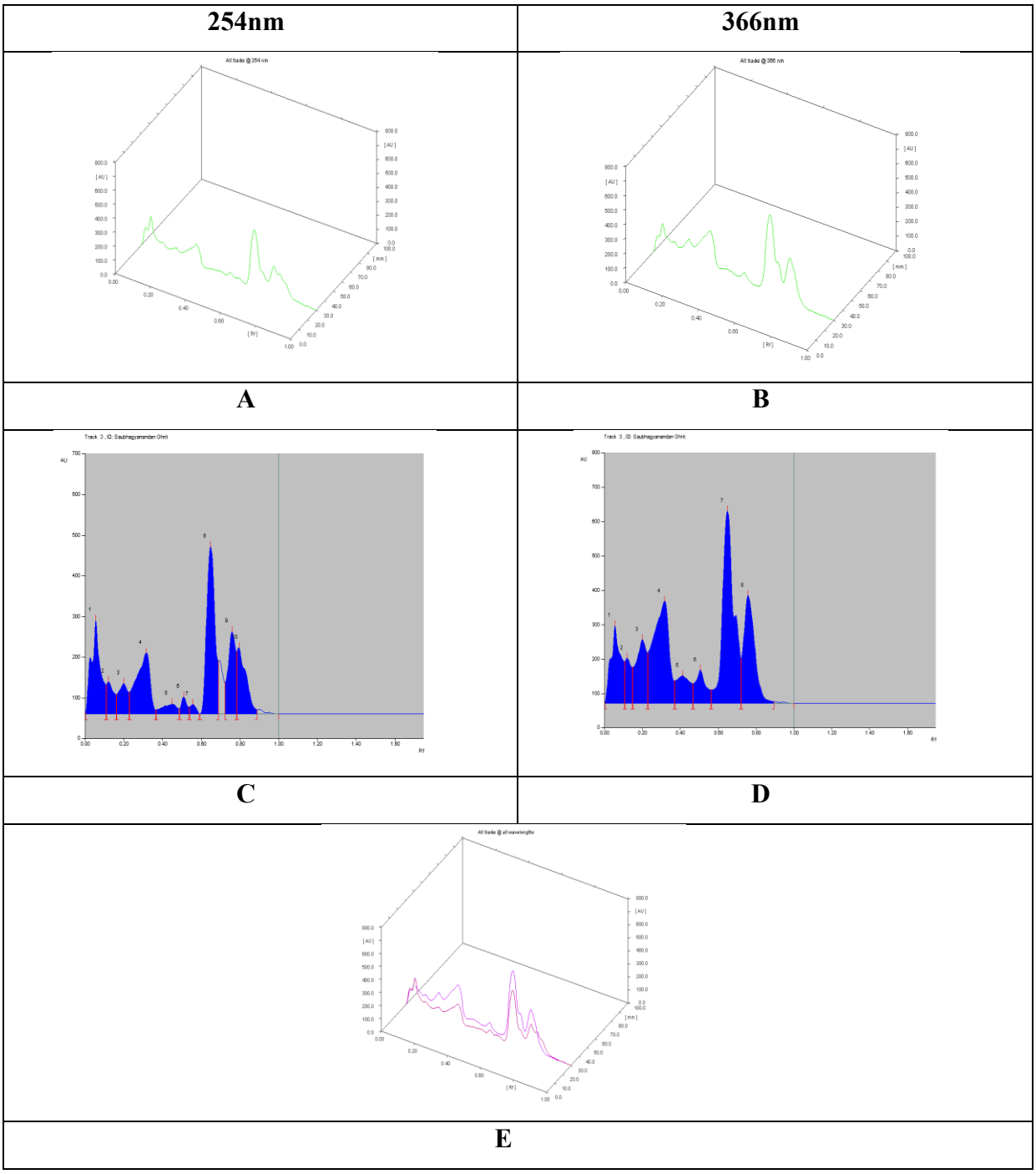

Figure 4: HPTLC profile of *Saubhagyanadana Ghrita*.

Here, **A** represents 3D graph at 254 nm, **B** represents 3D graph at 366 nm, **C** represents peak display at 254 nm, **D** represents peak display at 366 nm, **E** represents multiple wavelength 3D graph.

CONCLUSION

Phyto-chemical evaluation of *Ghrita* illustrated the specific characters of ingredients which were used in the preparation. Physico-chemical profile is an important parameter for quality control and assurance. In the present work, the obtained results were found within prescribed limits. For the first time, pharmacognostical, pharmaceutical and analytical profile of *Saubhagyanadana Ghrita* was established. On

the basis of the observations and results, obtained from the above methods adopted, this study may be used as standard in the further quality control researches.

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