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Pharmacognostical and analytical study of *Kushmanda ghrita*, a polyherbal formulation, for pharmaceutical standardization

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ABSTRACT

Kushmanda Ghrita is a unique polyherbal *Ayurvedic* formulation recommended in the management of various psychological disorders mentioned in *Astanga Hridaya* and also in *Bhaishajya Ratnavali* and the formula given is the same, selected for present study. Aim of the study was to develop the pharmacognostical and physico-chemical profile of *Kushmanda Ghrita*. *Kushmanda Ghrita* was prepared as per classical methods in the Ayurvedic Pharmacy, I.P.G.T. and R.A., Gujarat Ayurved University, Jamnagar and analytical findings were systematically recorded. The samples were subjected to organoleptic, pharmacognostical analysis and physico-chemical analysis. Pharmacognostical study containing both macroscopic and powder microscopy of raw drugs of *Kushmanda Ghrita* and Physico-chemical analysis of the finished product were carried out, to evaluate the quality of the formulation. Specific gravity of the formulation was 0.9205w/w, loss on drying at 110⁰ c temperature was 1.24%w/w, refractive index at 40⁰ c was 1.473, HPTLC was carried out after organizing appropriate solvent system in which maximum 11 spots at 256 nm and 4 spots at 356 nm were distinguished. Results are further used for any other research works.

Keywords: *Kushmanda Ghrita*, HPTLC, Pharmacognosy, Physico-chemical analysis.

INTRODUCTION

Analysis is very important for drugs as because it deals with life. Drug quality starts from identification, way of collection, in process during preparation and finished product etc.

In the present study, the *Kushmanda Ghrita* is subjected to Pharmacognostical and Pharmaceutical analysis.

Kushmanda Ghrita has been described in *Astanga Hridaya*, in the context of '*Apasmara Pratishedha*' i.e. *Dhi-vak-Swara pradam* ^[1] (it improves intellect, word and voice). It contains *Kushmanda swarasa*, *Yashtimadhu kalka* and *Go ghrita* ^[2].

Though *Kushmanda* has been repeatedly mentioned and appreciated for its '*chetovikaranasanam*' ^[3] (Psychological disorder) due to its *medhya* (nootropic) effect remains untouched. *Yashtimadhu* is included in main *Medhya rasayanas* (nootropic drugs) by *Acharya Charaka* ^[4] and it is commonly used for treatment of mental illness. *Ghrita* is generally used in the psychic disorder and seem to improve the faculties of mind. *Ghrita* is considered as the best "*sneha dravya*" because of its speciality ^[5] i.e. *Samskarsya anuvartanam* (the continuance of refinement) means *Ghrita* carries the properties of drug without leaving its own inherent properties ^[6].

Ayurveda is one of the oldest treatises of Indian medicine where medicines are from plant, animal and mineral origin. The efficacy of the *Ayurvedic* medicines depends on the genuine raw materials. So, proper identification of medicinal plant is most important. If the plants are adulterated then effective result of prepared medicine cannot give desirable therapeutic results.

Preliminary organoleptic features and results of microscopy were verified and all the ingredients were proved to be authentic. Quality of pharmaceuticals is based on pharmacopeia specifications.

MATERIALS AND METHODS

Collection, Identification and Authentication of raw drugs

Ripened fresh *Kushmanda* [*Benincasa hispida* (Fig.1)] was collected during middle of January, 2018, from the local marketing yard, Hapa, Jamnagar. Other ingredients of *Kushmanda Ghrita* (Table 1) were procured from Pharmacy, Gujarat Ayurved University. All the raw drugs were identified and authenticated in the Pharmacognosy laboratory, I.P.G.T. and R.A., Gujarat Ayurved University, Jamnagar and *Kushmanda Ghrita* was prepared in Pharmacy of Gujarat Ayurved University, Jamnagar, as per SOP (standard operative procedure) procured by pharmacy.

Table 1: Ingredients of *Kushmanda Ghrita*

Name	Botanical name	Part used	Form	Part
<i>Kushmanda</i>	<i>Benincasa hispida</i>	Ripen fruit	Swarasa	18 litres
<i>Yashtimadhu</i>	<i>Glycyrrhiza glabra</i>	Root	Powder	250 gms
<i>Go-Ghrita</i> (cow ghee)	-	-	-	1kg

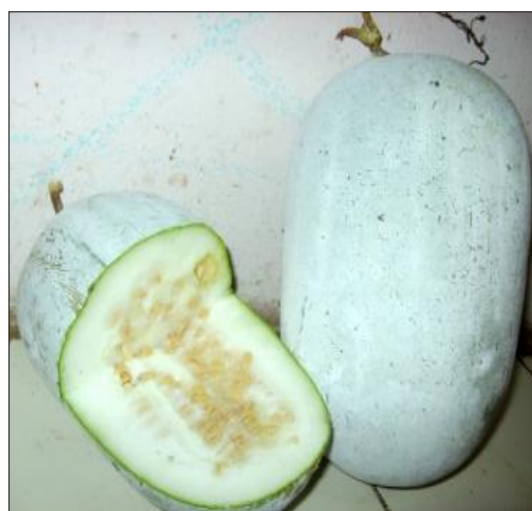


Figure 1: Fresh fruit of *Benincasa Hispida*

Method of preparation of *Kushmanda Ghrita*

Kushmanda swarasa was extracted by grinding machine from ripen *Kushmanda*. In a large vessel *Go-ghrita* was poured, when it was liquefied under moderate flame, *Kalka* of *Yashtimadhu* was added in *Kushmanda swarasa*, followed by additional *Kushmanda swarasa* was added. To get the final product, the contents were subjected to heat till ‘*Sneha Siddhi Lakshanas*’ were observed [7].

Pharmacognostical evaluation

The formulation was identified and authenticated and powder microscopy was done in the pharmacognosy laboratory, I.P.G.T. and R.A., GAU, Jamnagar, Gujarat, India. The study was included organoleptic evaluation and microscopic evaluation [8]. They are stored according to SOP (standard operational procedure) of WHO (World Health Organisation) guidelines [9].

Microscopic Study

Fine powder of *Kushmanda* fruit and *Yashtimadhu* was taken then examined under microscope without staining for the observation of cellular materials, then stained with Phloroglucinal and conc. HCl [10] for the lignified characters. Raw drugs were separately studied under microscope; the microphotographs of diagnostic characters were taken by using Carl zeiss trinocular microscope [11].

Organoleptic Study

Kushmanda Ghrita was evaluated for organoleptic characters like taste, odour and color, touch [12].

Physico-chemical analysis

Physico-chemical parameters of *Kushmanda Ghrita* like loss on drying at 110°C, Acid value, Iodine value, Refractive Index at 40°C, Saponification value, Specific gravity, HPTLC were determined as per the API guideline [13].

HPTLC

Methanol extract of *Kushmanda Ghrita* was used for High performance thin layer chromatography (HPTLC) study. Methanol extract of was spotted on *Kushmanda Ghrita* pre-coated silica gel GL60254 aluminium plate as 10mm bands by means of a CAMAG Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene (9ml) and ethyl acetate (1ml) was used for *Kushmanda Ghrita* as a mobile phase. The development time was 30 minutes. After development, Densitometry scanning was performed with a CAMAG TLC scanner III in reflectance absorbance mode at 254nm and 366 nm under control of Win CATS software [14].

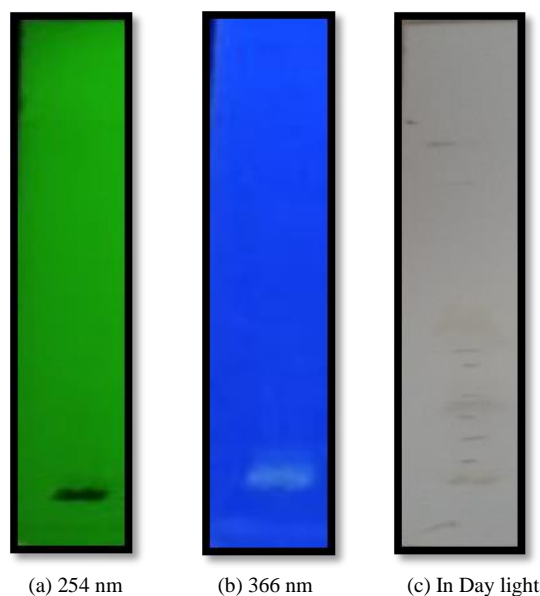


Figure 2: HPTLC Fingerprint Images of *Kushmanda Ghrita* (a) at 254 nm (b) at 366 nm (c) after spray in day light

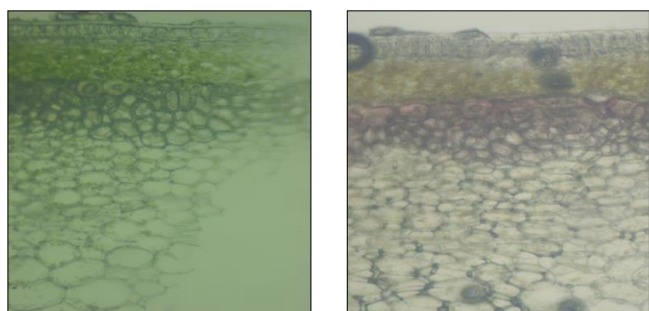
Observations and results

Macroscopic Evaluation

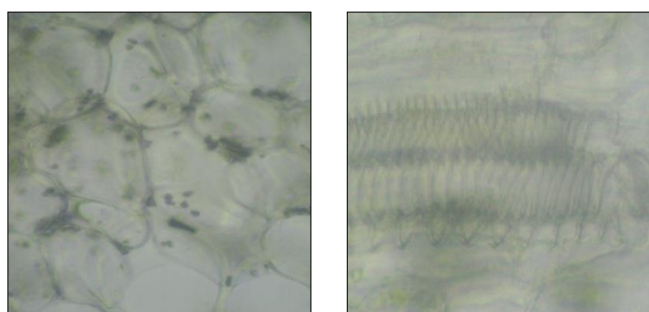
- Dried cut pieces of the *Kushmanda* fruit are irregular in shape with thick, smooth, glossy, curved, pericarp; pale whitish brown pithy pulp of the mesocarp. Taste is slightly sweet; odor characteristic.
- Dried peeled off brownish powder of *Yashtimadhu*.

Microscopic Evaluation

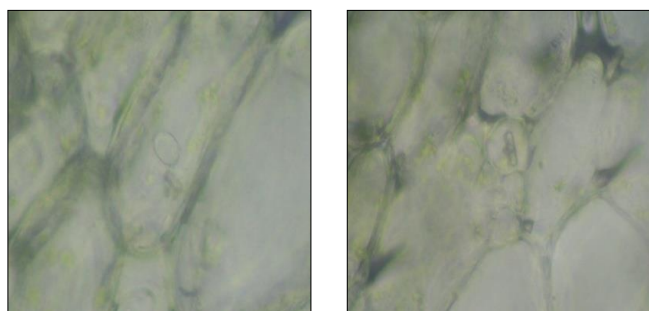
Matured fruit shows cuticularised epicarp consisting of single layered, squared or slightly tangentially elongated cells of epidermis, outer tangential walls of epidermis, outer tangential walls of epidermis thickened and cuticularised. A few epidermal cells divide peripherally and become 2 to 3 layers. Mesocarp has a heterogeneous structure. Perisperm cells, silica deposition stone cells were found. After staining lignified group of stone cells, fragments of lignified epicarp cells were found. (Plate 1, Fig. A-G).



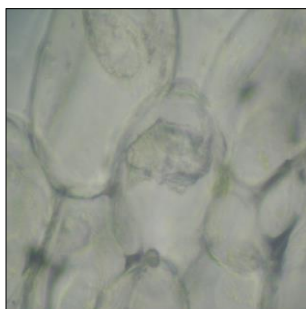
A. T.S. with epicarp, mesocarp and endocarp **B.** Epicarp followed by stone cell layer



C. Parenchyma cells with prismatic crystals **D.** Mesocarp cells with vascular strands



E. Parenchyma cells with simple starch grains **F.** Parenchyma cells with rod shaped crystal



G. Parenchyma cells with fragment of cystolith

Plate 1: Microscopic Photographs of Trans Section of *Kushmanda*

Powder Microscopy

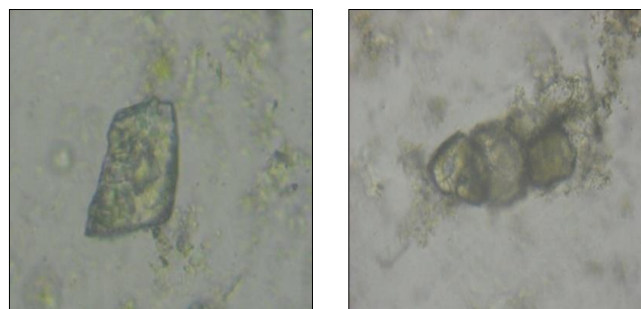
Organoleptic characters

Colour, odor, taste and touch were recorded and depicted in the Table 2.

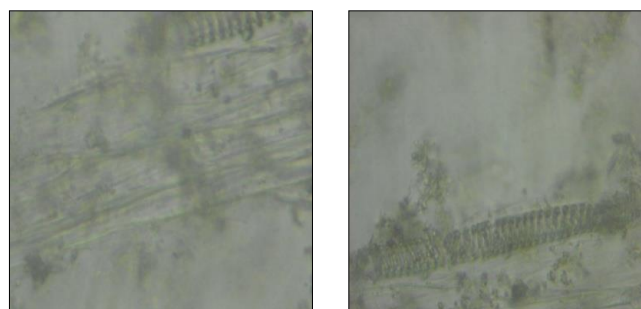
Table 2: Organoleptic characters of powder of *Kushmanda* fruit

Sl. No.	Character	Observation
1	Color	Whitish dull yellow
2	Nature	Coarse
3	Taste	Slightly Sweetish
4	Smell	Slightly Aromatic

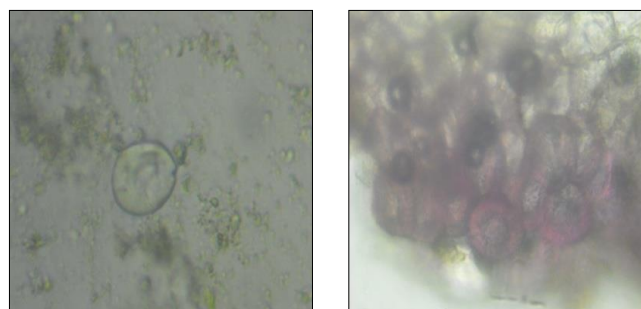
Diagnostic characters under the microscope observed before staining are stone cells, group of stone cells, perisperm cells, group of fibers, fragment of spiral vessels, silica deposition, simple starch grain, epical cells; after staining lignified group of stone cells, fragments of epical cells showed in (Plate 2, Figs: A-G).



A. Stone cells **B.** Group of Stone cells



C. Group of fibres with annular vessel **D.** Fragment of Fibres



E. Simple starch grain **F.** Group of Lignified pitted stone cells

Plate 2: Powder Microscopic Photographs of *Kushmada*

Macroscopic Study of *Yashtimadhu* (*Glycyrrhiza glabra*)

The Organoleptic characters of *Yashtimadhu* (*Glycyrrhiza glabra*, Linn.) showed in Table 3.

Table 3: Organoleptic Characters of *Yashtimadhu* Powder

Sl.No.	Character	Observation
1	Color	Brownish yellow
2	Nature	Coarse
3	Taste	Sweet
4	Odour	Aromatic

Microscopic study of *Yashtimadhu*

Microscopic study of *Yashtimadhu* powder before staining border pitted vessels, dark brown content, simple and compound starch granules, fragments of annular vessels, group of fibres, simple starch grain with hilum, rhomboidal crystal, group of crystal fibres, group of lignified fibres, light brown content, fragment of cork cells: after

staining group of lignified fibres, fragments of lignified border pitted vessel, group of lignified crystal fibres, group of lignified stone cells, fragment of lignified annular vessels, fragment of cork cells found, showed in (Plate-3, Fig: A-F).

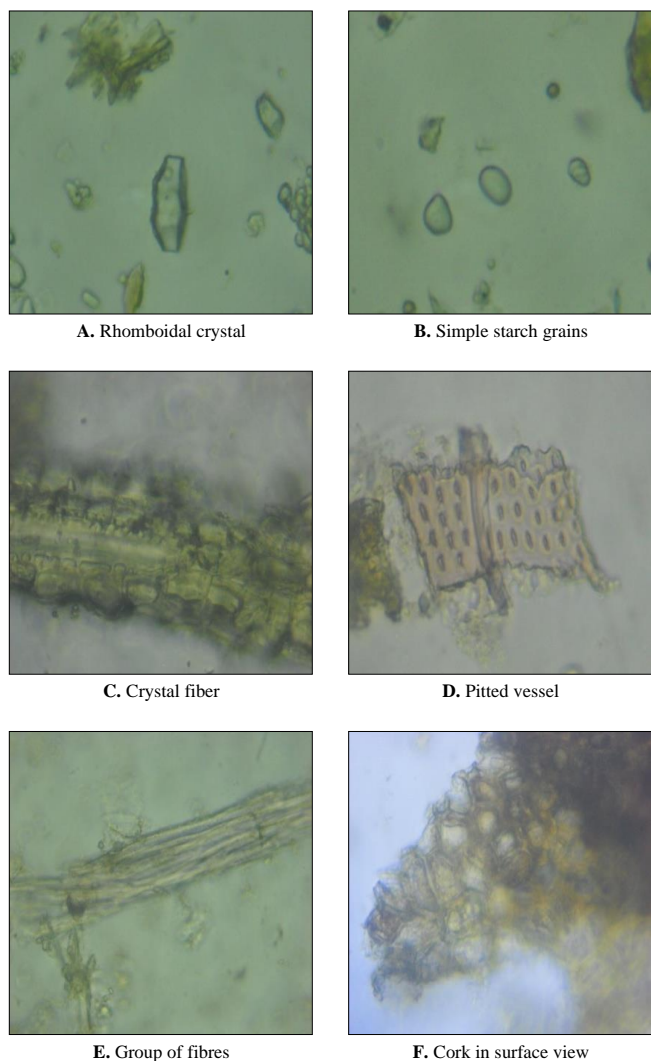


Plate 3: Powder Microscopic Photographs of *Yeshthimadhu*

High Performance Thin Layer Chromatography (HPTLC)

Preparation of sample solution

The *Kushmanda Ghrita* sample was absorbed on silica gel. The mixture was extracted with hexane. Hexane fraction was discarded.

Chromatographic conditions

HPTLC was performed as per the guideline provided by API. Methanolic extract of drug sample was used for the spotting. HPTLC was performed using Petroleum ether: Di-ethyl ether: Acetic acid= 9:1:0.1 v/v solvent systems. The colour and Refractive values of resolved spots were noted. High-Performance Thin Layer Chromatography study Methanol extract of Sample was spotted on pre-coated silica gel GF254 aluminum plate as 6 mm bands, 5 mm apart and 1 cm from the edge of the plates, by means of a Camang Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Petroleum ether (7ml), Di-ethyl ether (2ml), (0.5ml) was used as the mobile phase. After development, Densitometric scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (v1.2.1 camag). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm.

Sample preparation

0.1ml of sample was taken and diluted with 1ml of hexane and then subjected to chromatography. There after prechromatographic derivatisation was done with alcoholic KOH.

OBSERVATION AND RESULTS

Organoleptic characters of *Kushanda Ghrita*:

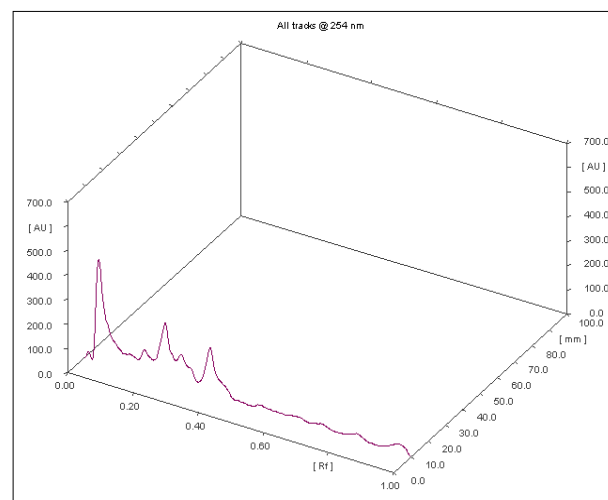
Organoleptic characters contents of *Kushanda Ghrita* like colour, taste, touch and odor were recorded and showed in (Table 4).

Table 4: Organoleptic Characters of *Kushanda Ghrita*

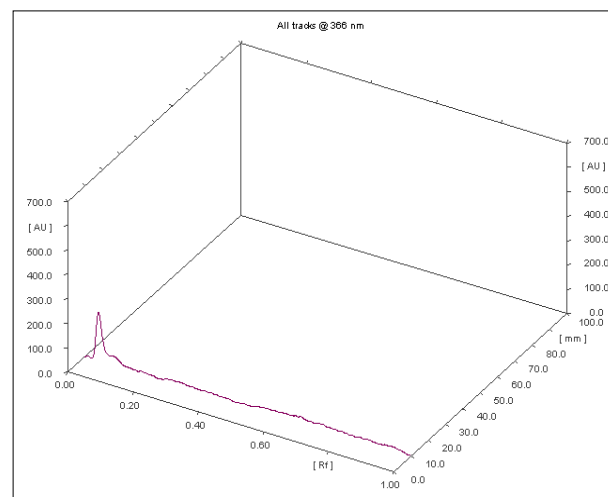
Sl. No.	Character	Observation
1.	Color	Golden brown
2.	Touch	Smooth
3.	Taste	Sweetish
4.	Odor	Sweetish

Pharmaceutical evaluation

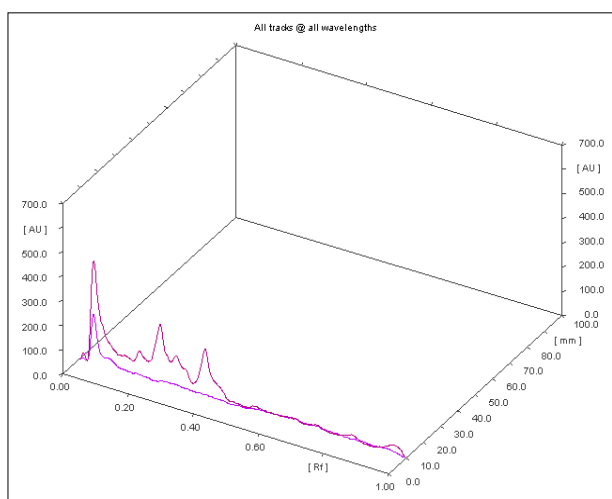
Physico-chemical analysis of *Kushanda Ghrita* showed on Table 5 Maximum R_f of *Kushanda Ghrita* in HPTLC showed in Table 6. Fig. 3 (a) 254nm 3D (b) 366 nm 3D (c) spectral comparison at R_f of *Kushanda Ghrita*. Plate 4 showed peak display of *Kushanda Ghrita* at 366 nm 4 spots and at 254 nm 11 spots.



a

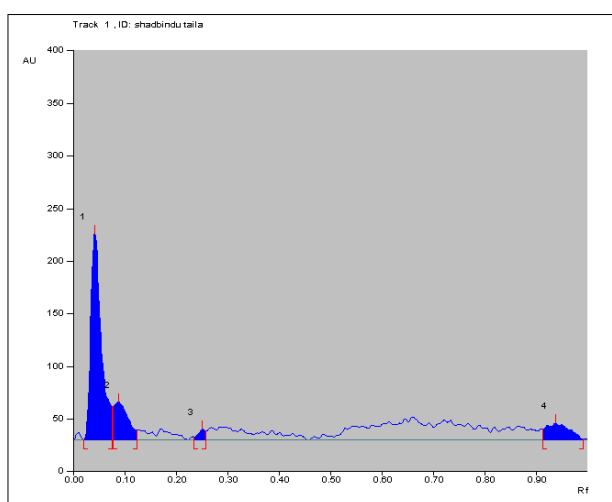


b

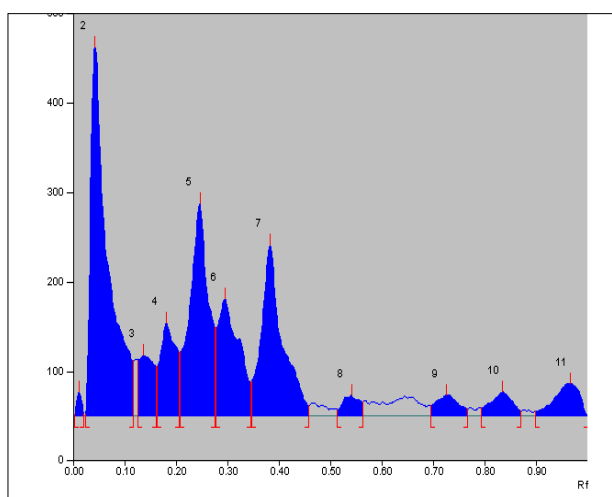


c

Figure 3: a. 254 nm 3D, b. 366 nm 3D, c. Spectral comparison at R_f



(a) Peak display at 366 nm



(b) Peak display at 254 nm

Plate 4: Peak display of *Kushmanda Ghrita* at 366 nm 4 spots and 254 nm 11 spots

Table 5: Physico - Chemical Analysis of *Kushmanda Ghrita*

S No.	Test	Result
1	Loss on drying at 110 ^o C	1.24% w/w
2	Specific Gravity at room temperature	.9205 w/w
3.	Refractive Index at room temperature	1.43
4.	Acid value	4.63 w/w
5.	Saponification	65.89 w/w
6.	Iodine value	2.92 w/w

Table 6: Maximum R_f of *Kushmanda Ghrita* in HPTLC showed

Sample name	254nm		366nm		After derivatisation	
	No. of spots	R _f values	No. of spots	R _f values	No. of spots	R _f values
<i>Kushmanda Ghrita</i>	11	0.01,0.04,0.14, 0.18, 0.25, 0.29, 0.38,0.54,0.72, 0.83,0.97	4	0.04, 0.09, 0.25, 0.94	7	0.12, 0.18, 0.21, 0.25,0.30,0.32, 0.92

DISCUSSIONS

Pharmacognostical study reveals authentication of individual raw drugs of *Kushmanda Ghrita* and was cross verified. Stone cells, silica deposition, fragment of spiral vessels of *Kushmanda* and border pitted vessels, simple starch granule starch granule with hilum, rhomboid crystal, group of crystals and fragment of cork cells, group of lignified stone cells were observed in ingredients. All the physico-chemical parameters, acid value, saponification value, R_f value, iodine value, specific gravity analyzed were within normal referential range. In HPTLC spots were detected at different R_f value All the results showed that the prepared *Ghrita* formulation was not adulterated and rancid and the quality of *Kushmanda Ghrita* has been standardised.

CONCLUSION

Pharmacognostical study finding confirms the ingredients present in *Kushmanda Ghrita* and support the intended action of the formulation. Under densitometer at 254nm 11 peaks and under 366 nm 4 peaks were found and after spray 7 peaks were found. It is showing that the finished products meet maximum qualitative standards. The results of this study may be used as the reference standard in further research as because previously no analytical study has not been reported, probably it is the first analytical as well as pharmacognostical study of *Kushmanda Ghrita*.

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