WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 921-930. Research Article ISSN 2277– 7105

INGREDIENTS IDENTIFICATION, PHYSICO-CHEMICAL AND HPTLC ANALYSIS OF *VAJRAK GHRITA*– A POLYHERBAL FORMULATION

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Article Received on
27 Sept. 2017,

Revised on 17 Oct. 2017,
Accepted on 07 Nov. 2017

DOI: 10.20959/wjpr201715-10141

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ABSTRACT

Background: *Vajrak Ghrita* (VG) is mentioned in *Ayurvedic* classics as a therapeutic formulation to treat *Kushtha* (Skin diseases). The *Ghrita* was prepared with respective pharmacognostically identified ingredients those are *Vasa* (*Justicia adhatoda* Linn.), *Guduchi* (*Tinospora cordifolia* Miers.), *Nimba* (*Azadirachta indica* A. Juss), *Patola* (*Luffa acutangula* Linn.), *Kantakari* (*Solanum surrattense* Burm.f.), *Karanj* (*Pongamia pinnata* Linn.), *Triphala.* The base was *Go-ghrita.* All the ingredients have *Kushthaghna* properties. **Materials and Methods:** Powders of all ingredients were evaluated for their pharmacognostic study and finished product which is VG was evaluated for pharmaceutical analysis. **Results:** Some typical microscopic characters were found of *Vasa, Guduchi, Nimba, Patola, Kantakari, Karanj, Triphala.* Results obtained in pharmaceutical parameters of VG, Acid value 3.4, Refractive Index 1.4710, Iodine value 28, Saponification Value 296.47, Specific Gravity 0.9254, Loss on drying 0.15, pH value 3.5 are within limit mentioned by Ayurvedic Pharmacopoeia of India. High performance thin layer chromatography profile of VG powder showed similarities in number of spots. **Conclusion:** From the study, data developed can be espoused for laying down the standards for VG.

KEYWORDS: HPTLC**,** Pharmacognostical, Pharmaceutical analysis, *Vajrak Ghrita.*

INTRODUCTION

Acharya Charaka has mentioned *Ekkushtha* under the title of *Kshudra Kustha*. *Ekkushtha* consists of the signs and symptoms i.e. *Aswedana, Mahavastu* and *Matsyashakalopam Avastha*[1] which can be compared with Psoriasis and hence it has been taken as the analogue to Psoriasis in the present research work. Psoriasis is a chronic, autoimmune disease that appears on the skin. There is about 2.5% of whole world population today who are suffering from psoriasis.[2] Despite *Tridoshaja* origin of *Ekkushtha*, Charaka mentioned dominancy of *Vata* and *Kapha.* According to *Ayurveda* three types of *Chikitsa* are described by *Acharyas* among them *Shamana Chikitsa* is there. So *Vajrak Ghrita* (VG) was selected as *Shamana* for treatment of *Ekkushtha*.

VG contains *Vasa, Guduchi, Nimba, Patola, Kantakari, Karanj* and *Triphala* (Table 1) which was first mentioned by *Acharya* Vagbhatt.[3] During the last decades, herbal medicines pointed out in *Ayurveda* are getting gratitude globally. Maintaining the quality standard of a polyherbal formulation is a challenging task. Available data concerning the scientific evaluation of VG are none. In view of severe undesirable side effects of drug, there is growing focus to follow systematic research methodology and to provide scientific basis for the traditional herbal medicines. The first step for scientifically based research is to provide quality standardization of drug. With this background the present study was done to establish the authenticity of all the ingredients of VG. VG analyzed pharmacognostically by dry samples of ingredients of *Vajrak Ghrita* macroscopically and microscopically. Preliminary analyze the *Vajrak Ghrita* by using different physico-chemical parameters and to develop the HPTLC (High Performance Thin Layer Chromatography) profile of *Vajrak Ghrita.*

MATIRIALS AND METHOD

Collection of the Raw Drugs

All the raw drugs of *Vajrak Ghrita* were collected from Pharmacy, Gujarat Ayurveda University (GAU), Jamnagar, India and all these drugs were identified and authenticated in Pharmacognosy Laboratory, Institute for Postgraduate Teaching and Research in Ayurveda (IPGT & RA), GAU, Jamnagar, India. Ingredients of *Vajrak Ghrita* are summarized at [Table 1].

Table 1 Ingredients of *Vajrak Ghrita.*

Sr. No.	Drug	Botanical Name	Part	Part used
1	*Vasa*	*Justicia adhatoda* Linn.	1 part	*Patra* (Leaves)
2	*Guduchi*	*Tinospora cordifolia* Miers.	1 part	*Panchang* (Whole plant)
3	*Nimba*	*Azadirachta indica A.* Juss.	1 part	*Tvak* (Bark), *Patra*
4	*Patola*	*Luffa acutangula* Linn.	1 part	*Mula* (Root)
5	*Kantkari*	*Solanum surrattense* Burm.f.	1 part	*Panchang*
6	*Karanj*	*Pongamia pinnata* Linn.	1 part	*Beeja* (Seed)
7	*Haritaki*	*Terminalia chebula* Retz.	1/3 part	*Phala* (Fruits)
8.	*Bibhitaki*	*Terminalia Bellirica* Roxb.	1/3 part	*Phala* (Fruits)
9.	*Aamlaki*	*Emblica officinalis* Gaertn.	1/3 part	*Phala* (Fruits)
10.	*Go-ghrtia*	Cow Ghee	QS	-

Microscopical evaluation of powdered raw drugs of *Vajrak Ghrita*

It is possible to analyse the finished products for the pharmacognosy i.e. Compound formulations like *Vati* (tablet), *Churna* (powder), *Kalka* (paste) etc., but it is difficult to analyse the *Ghrita* to find out the cellular level of raw drugs. In this study as *Vajrak Ghrita* was made from *Kwatha* (decoction) & *Kalka* (paste) of above mentioned drugs (Table 1), thus raw drugs powder was studied separately with and without staining. The micro pictures were taken under Carl zeis microscope attached with camera.[4]

Preparation of *Vajrak Ghrita*

Vajrak Ghrita was prepared in RSBK *(Rasashastra* and *Bhaishajya Kalpana)* department, IPGT & RA, GAU, Jamnagar, India. All identified drugs were washed and dried properly. *Kwatha* (decoction) was prepared by adding 8 times water in equal amount of all drugs and then it was boiled in low flare to decrease it to 1/4th of total water.[5] *Kalka* (paste of powder) was prepared by adding adequate amount of water in above mentioned drugs. For preparation of *Vajrak Ghrita* 1: 4: 16 of *Kalka*, *Ghrita* and *Kwatha* respectively were taken as per classical reference.[6] After preparation of *Kalka* and *Kwatha*, *Ghrita* was measured and poured into a vessel with thick base on medium flare. The *Kwatha* and *Kalka* were also poured into the vessel and the mixture was boiled in medium flame with continuous stirring and monitoring of *Paka*. The boiling was stopped and the *Ghrita* was sieved by using a washed and dried white filter cloth when *Madhyama Paka*[7] was attained.

Organoleptic study of prepared drug

Organoleptic studies of prepared *Vajrak Ghrita* are endangered for various sensory characteristics like odour, colour etc. were carefully distinguished down.

Physico-chemical analysis

Physico-chemical analysis of *Vajrak Ghrita* was done by using various standard physico-chemical parameters such as Acid value[8], Refractive Index value[9], Saponification value[10], Iodine value[11] and Specific gravity[12] at Pharmaceutical chemistry laboratory, IPGT and RA, Jamnagar, India. Physico-chemical analyses were carried out by following standard procedure mentioned in API (Ayurvedic Pharmacopeia of India).

6. HPTLC (High Performance Thin Layer Chromatography) evaluation[13]

A. Sample Details

Vajrak Ghrita.

B. HPTLC Conditions

0.1 ml *Vajrak Ghrita* with 1 ml Hexane and it was used and it was spotted as 5 microlitre.

C. Stationary Phase

Merk, 1.05548.0001, TLC Silica gel 60 F254, 10x10 cm Aluminium sheet.

D. Mobile Phase

Petroleum ether: Diethyl ether: Acetic acid (9:1:0.1 v/v).

E. Development

CAMAG 20 x 20 cm Twin trough chamber.

F. HPTLC Instrumentation

CAMAG Linomat 5, CAMAG TLC Scanner 3, CAMAG Reprostar 3.

G. Derivatization

10% sulphuric acid reagent.

Sample was prepared by diluting 0.1 ml *Vajrak Ghrita* with 1 ml Hexane and it was used for spotting. Prepared sample of *Vajrak Ghrita* was spotted on pre-coated silica gel aluminium plate as 6 mm bands by means of a CAMAG Linomat V sample applicator fitted with a 100 µL Hamilton syringe. Then alcoholic KOH was applied on same spotted area and plate was heated at 110 C on TLC plate heater for 10 minutes. Petroleum ether: Diethyl ether: Acetic acid (9:1:0.1 v/v) was used for *Vajrak 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 254 nm and 366 nm under control of Win CATS software (V1.3.4 CAMAG). Then the plate was dipped in 10% H_2SO_4 followed by heating and then visualized in day light. The Rf values and colour of resolved spots were noted.

OBSERVATIONS AND RESULTS

Microscopic Characters

Powder microscopy characters of individual herbal drugs of *Vajrak Ghrita* were observed as below [Table No. 2] and microphotographs are placed at respective plate. [Plate No. 1].

Table No. 2 Microscopic character of drugs.

Sr. No.	Drug	Identified Microscopic Characters
1	*Vasa*	Compound starch, pitted vessels, Simple Trichomes
2	*Guduchi*	Cork Cell, Collenchyma, Fragments of pitted vessels
3	*Nimba*	Lignified Fibres, Prismatic Crystal, Unicellular Trichomes
4	*Patola*	Annular & Spiral Vessels, Septet Fibres, Tannin Content
5	*Kantkari*	Stone Cells, Multibranch Trichome with Fibres, Starch Grain with Tannin
6	*Karanj*	Cork cell in surface view, Crystal Fibres, Starch Grain with Tannin
7	*Triphala*	Rosett Crystal, Pitted Screoid and Vessels, Lignified Fibre, Mesocarp, Prism stone

Organoleptic Characters

Organoleptic characters of prepared *Vajrak Ghrita* carefully observed and distinguished as below. [Table No. 3].

Table No 3 Organoleptic study of prepared drug.

Parameter Studied	Observations
Colour	Opaque Brown
Odour	Slightly Bitter
Consistency	Slightly thick & Single thread

Physico-chemical results

Physico-chemical findings of prepared *Vajrak Ghrita* are given in below table. [Table No. 4].

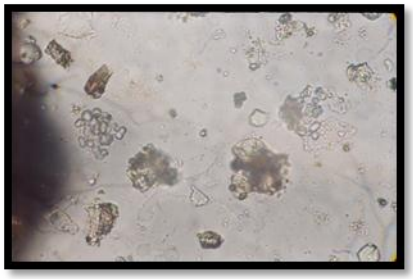
Table No. 4. Physico-chemical findings of prepared *Vajrak Ghrita*.

Parameter studied	Results
Acid value	3.4 % w/w
Refractive Index	1.4710
Iodine value	28% w/w
Saponification Value	296.47% w/w
Specific Gravity	0.9254
Loss on drying	0.15% w/w
pH value	3.5

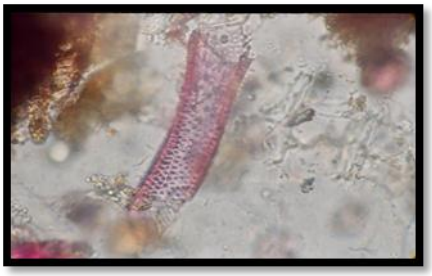
Table No. 5: Rf values.

Visualize under short UV (254 nm)		Visualize under short UV (366 nm)	
No. of spot separated	**Rf Values**	**No. of spot separated**	**Rf Values**
11	0.03,0.07,0.13,0.24,0.26,0.37,0.52, 0.59,0.78,0.94,0.98	04	0.03,0.13,0.52,0.98

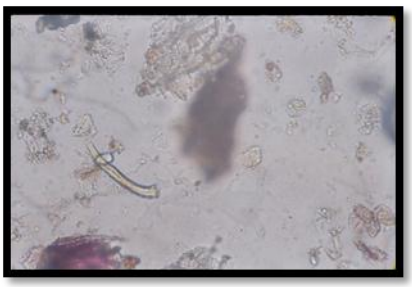
Plate No. 1.



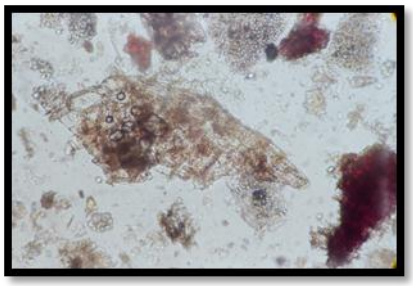
Compound Starch (*Vasa*).



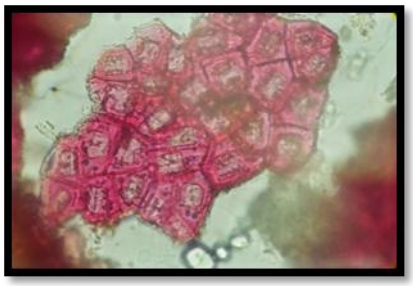
Pitted vessels (*Vasa*).



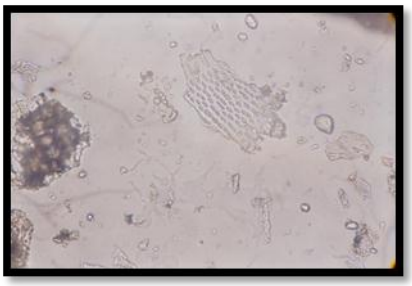
Simple Trichomes(*Vasa*).



Cork Cell (*Guduchi*).

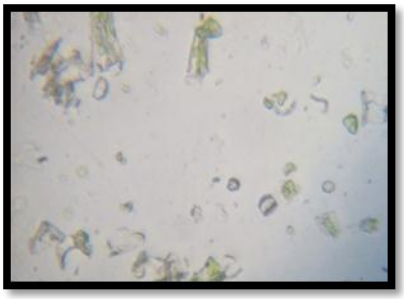


Collenchyma (*Guduchi*).

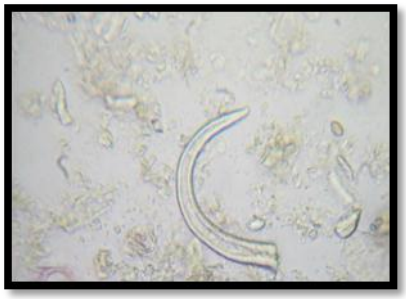


Fragments of pitted vessels (*Guduchi*).

Lignified Fibres (*Nimba*).

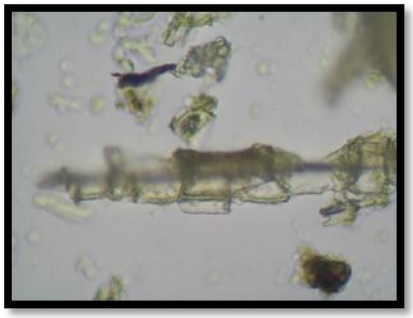


Prismatic Crystal (*Nimba*).

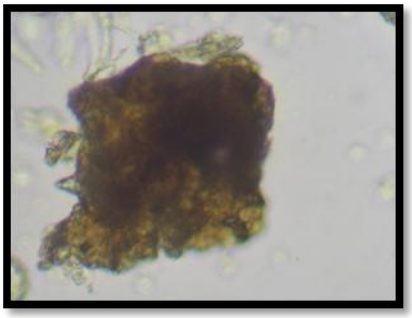


Unicellular Trichomes (*Nimba*).

Annular & Spiral Vessels (*Patola*).



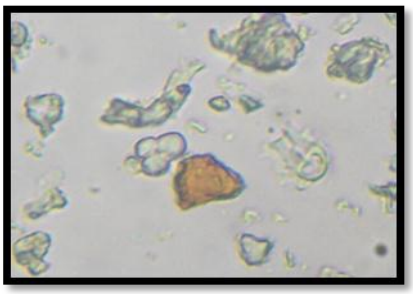
Septet Fibres (*Patola*).



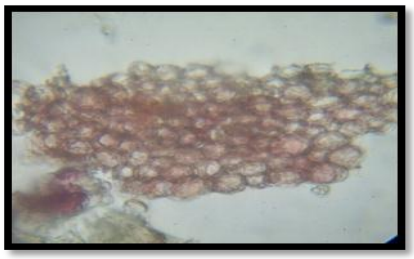
Tannin Content (*Patola*).

Stone Cells (*Kantkari*).

Multibranch Trichome with Fibres (*Kantkari*).

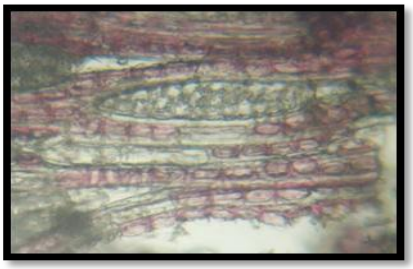


Starch Grain with Tannin (*Katakari*).

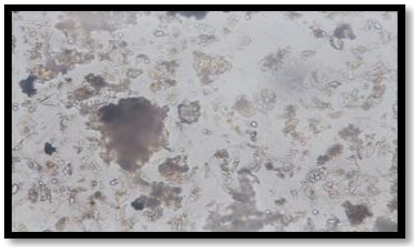


Cork cell in surface view (*Karanj*).

Crystal Fibres (*Karanj*).



Medullary Rays (*Karanj*).

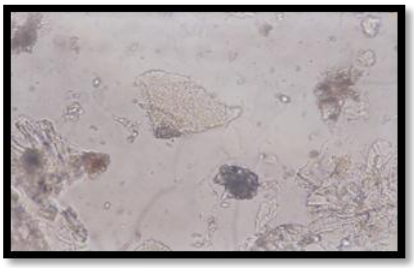


Rosett Crystal (*Haritaki*).

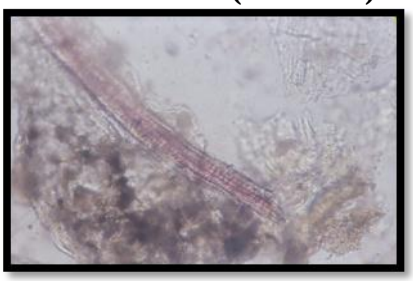
Pitted Screoid (*Haritaki*).

Pitted Vessels (*Bibhitaki*).

Lignified Fibre (*Bibhitaki*).

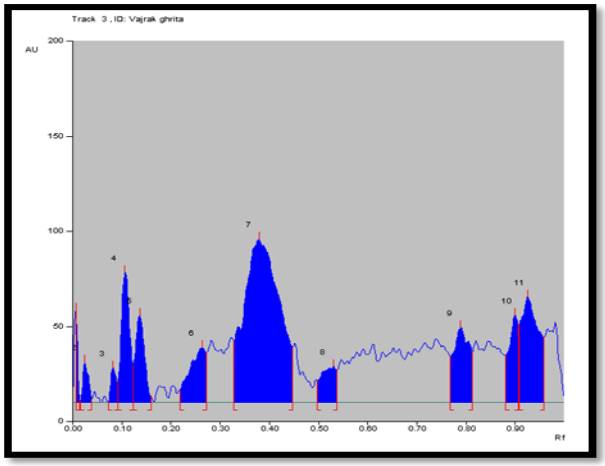


Mesocarp (*Aamlaki*).

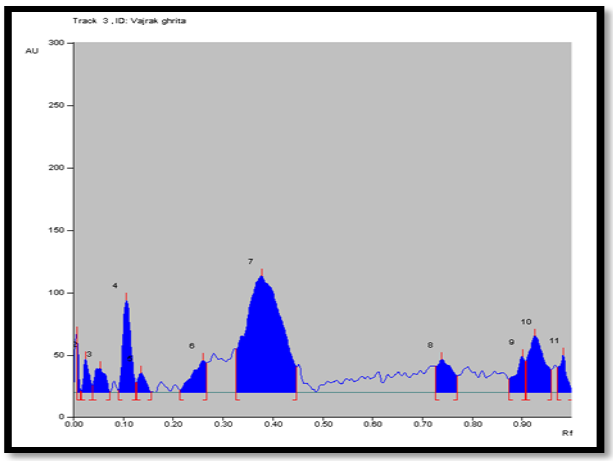


Prism stone (*Aamlaki*).

Plate No. 2: Densitogram at 254 nm and 366 nm.

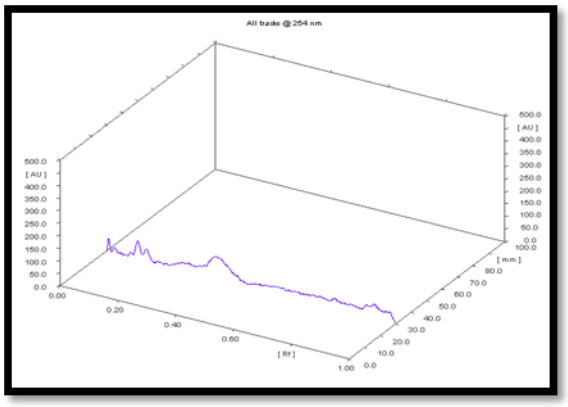


At 254 nm.

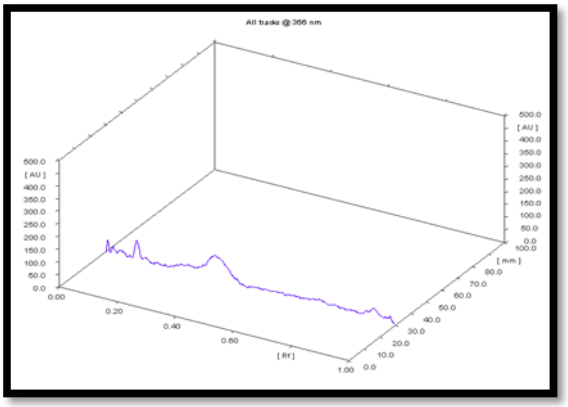


At 366 nm.

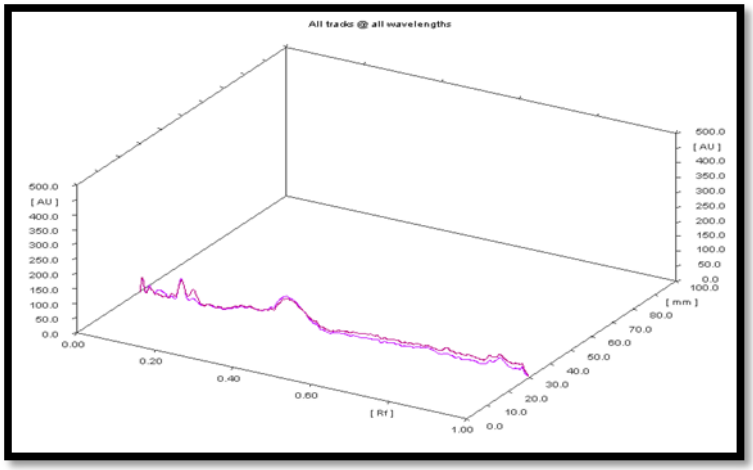
Plate No. 3: Three dimensional (3D) Densitogram At 254nm, 366nm & Comparison at 254nm & 366nm.



At 254 nm

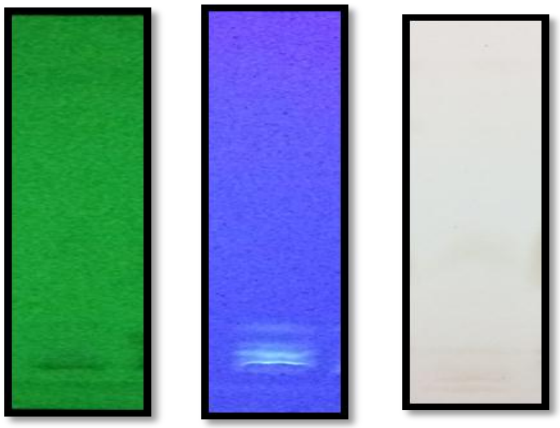


At 366 nm



Comparison at 254 nm & 366 nm.

Plate-4: HPTLC finger prints at 254 nm and 366 nm.



At 254 nm **At 366 nm** **After Spray**

❖ DISCUSSION

The Pharmacognostical study exposes authentication of individual raw drugs of *Vajrak Ghrita* and it is cross verified in *Ayurvedic* Pharmacopeia of India (API). The pitted vessels,

oil globules, rhomboidal crystal, starch grains, prismatic crystals, fibres etc. were observed in ingredients. Quality control parameters like specific gravity, saponification value are standard for any fat or oil. Similarly, when oil-fats become rancid, triglycerides are converted into fatty acids and glycerol causing an increase in acid value. Iodine value and refractive index values are suggestive of oxidation.[14] The oxidation levels of vegetable oils are important quality criteria in food chemistry because oxidation increases their toxicity by the formation of products such as hydroperoxides, aldehydes, ketones, etc.[15] All physico-chemical parameters; acid value, iodine value, saponification value, specific gravity, refractive index analyzed were almost near to the reference range as identified for *Vajrak Ghrita.*[16] In this study *Vajrak Ghrita* is well separated compact symmetrical bands in favour of chromophore sensitive component (Sterol, phytosterol, stigmasterol etc.) indirectly due to prechromatographic derivatization of oil sample directly. By visualization under short UV there were 11 spots and while under long UV exposure 4 spots (Table 5/Plate 2 & 3). Component represent by the Rf 0.03, 0.13, 0.52, 0.98 were common in both light exposure.

❖ CONCLUSION

It is concluded that the formulation meets maximum qualitative standards based on physico-chemical parameters. The separation pattern of VG is documented with help of prechromatographic derivative method in context of R_f & densitogram. The findings from this study will provide systemic evaluation and also serve as a master document to control the quality of *Vajrak Ghrita* formulation. The study results may be used as the reference standard in further research undertakings of its kind.

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