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MICROBIOLOGICAL, PHARMACOGNOSTICAL AND PHYTOCHEMICAL PROFILE EVALUATION OF MAHATRIPHALADI GHRITA: A COMPOUND AYURVEDIC FORMULATION

[1] AMEESHA R. SHRIGOD, [2] D. B. VAGHELA, [3] DEEPAK PAWAR [4] HARISHA C. R., [5] MEERA CHOLERA, [6] SHUKLA V. J.

*[1]2nd year PG Scholar, [2]Head and Associate Professor, [3]Assistant Professor, [4,5,6]Head
[1,2,3]Department of *Shalakya Tantra*.
[4]Pharmacognosy Lab.. [5]Microbiological Lab.
[6]Pharmaceutical chemistry Lab, I.P.G.T and R.A., Gujarat Ayurved University, Jamnagar, Gujarat
Email – [1] Ameeshashrigod@gmail.com

Abstract: **Aim and Objective:** *Mahatriphaladi Ghrita* is a multi-herbal Ghrita preparation which has *Tridoshashamak* and *chaksushya* properties mentioned in ancient texts. *Mahatriphaladi Ghrita* is a drug of choice for diseased condition like Myopia or Nearsightness which is the most common refractive error of the eyes and it has become more prevalent in recent years due to overuse of different gadgets. It was mainly aimed to develop the Microbiological, Pharmacognostical and phytochemical profile of Mahatriphaladi Ghrita for the extraction, screening and identification of medicinally active substances found in preparation. **Methodology:** Preliminary Microbiological studies, Pharmacognostical and phytochemical parameters, High-Performance Thin Layer Chromatography has been performed as per Standard method. **Result:** Microbiological study for Mahatriphaladi Ghrita revealed absence of any microbial contamination under smear and culture study in aerobic atmosphere after 90 days of sample been prepared. The Pharmacognostical study of ingredients of Mahatriphaladi Ghrita shows the presence of Rosette crystal, scleroids, stone cells and many more. Pharmaceutical analysis of *Mahatriphaladi Ghrita* showed that loss on drying 0.39%w/w, Saponification value 188%, Specific gravity 0.9126% (at 40degree Celsius), Refractive index 1.42%, Iodine value 44.343% and High-Performance Thin Layer Chromatography at 254 nm resulted into 9 spots and at 366 nm resulted into 6 spots respectively. These parameters of pharmacognosy and pharmaceutical analysis can be used as baseline for future. **Conclusion:** The standardization of *Mahatriphaladi Ghrita* are covered in the current study, additional important analysis investigations are required for the identification of all the active chemical constituents of the test drug to substantiate the clinical efficacy.

Key Words: HPTLC, *Mahatriphaladi Ghrita*, Microbiological, Pharmacognosy, Physicochemical.

1. INTRODUCTION:

Mahatriphaladi Ghrita[i] is a multi-herbal Ghrita preparation which has *Tridoshashamak* and *chaksushya* properties mentioned in ancient texts. *Mahatriphaladi Ghrita* is a drug of choice for various diseases of Eyes. According to *Charaka, Ghrita* is effective in subsiding *Pittaja* and *Vataja* disorders, it improves *Dhatus* and is over all boosters for improving *Ojas.* According to *Sushruta* along with above said properties it provides strength to eye sight. *Bhavaprakasha* has also described Ghrita as *Rasayana*, good for the eyes and provide shield like protection to body from various diseases.

Moreover, the use of herbal medicine has increased remarkably in line with the global trend of people returning to natural therapies. The growing use of botanicals (drug and other products derived from plants) by public if forcing moves to assess the health claims of these agents and to develop standard of quality and manufacture.

Microbiological analysis is performed for the estimation of the no. of viable aerobic micro-organism presence and for detecting the presence of designated microbial species in pharmaceutical substance. Pharmacognosy deals with the authentication and Quality assessment of crude plant and herbal material based on Macroscopic and Microscopic characters. Phytochemical has generally been used to describe plant compounds that are under research with unestablished effect on health. Standardization of herbal medicines is the process of prescribing a set of inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. An herbal preparation cannot be considered scientifically valid if the drug tested has not been authenticated and Characterized in order to ensure reproducibility in manufacturing of the product. Moreover, many dangerous and lethal side effects have recently been reported, including direct toxic effect, allergic reaction, effects from contaminants and interaction with herbal drug. On this background, Standardization is an

important step for the establishment of a consistent biological activity, a Consistent chemical profile, or simply a quality assurance program for production and manufacturing of an herbal drug.

2. MATERIALS AND METHOD:

- Plant material:

The raw drug materials were collected from the pharmacy department, IPGT & RA, GAU, Jamnagar.

INGREDIENTS OF MAHATRIPHALADI GHRITA:[ii]

Sr.no.	Drugs	Botanical name	Part used	Proportion
1.	DRAVA DRAVYA:			
	• *Triphala:* *Haritaki* *Amalaki* *Bibhitaki*	*Terminalia Chebula Retz.* *Phyllanthus Emblica Linn.* *Terminalia Belerica Roxb.*	Fruit	
	• *Bhringaraja*	*Eclipta Alba Hassk*	Whole	
	• *Vasa*	*Adhatoda Zeylanika Medic.*	Leaves	
	• *Shatavari*	*Asparagus Racemosus willd*	Roots	1 part each
	• *Guduchi*	*Tinospora Cordifolia willd.*	Rhizome	
	• *Amalaki*	*Phyllanthus Emblica Linn.*	Fruit	
	• *Aja dugdha*	*Goat milk*	---------	
2.	KALKA DRAVYA:			
	• *Triphala:* *Haritaki* *Amalaki* *Bibhitaki*	*Terminalia Chebula Retz.* *Phyllanthus Emblica Linn.* *Terminalia Belerica Roxb.*		1/8th part
	Pippali	*Piper Longum Linn.*		
	Draksha	*Vitis Vinifera Linn.*	fruit	
	Neelkamala,	Nymphaea Nouchali Linn.	Rhizome	
	Yashtimadhu	Glycyrrhiza Glabra Linn.	Root	
	Kshirakakoli	Fritillaria Hook. F	Root	1/8th part
	Mishri	Sugar candy	----------	
	Gambhari	Gmelina Arborea Roxb.	Root	
	Kantakari	Solanum Xanthocarpum sch.	whole	
	Go Ghrita	Coe ghee	----------	1 part

3. METHOD OF PREPARATION OF MAHATRIPHALADI GHRITA:[iii]

- Equal part of each Kwatha dravya mentioned above was taken in a stainless-steel vessel and made into Kwatha by soaking overnight in 8 times water to the dravya which was reduced to 1/4th by heating it on medium flame separately.

↓

- *Kalka* Dravya as mentioned above taken in equal quantity and made into bolus form (Kalka)

↓

- *Ghrita* will be taken in a stainless-steel vessel and heated mildly to remove any moisture. Then ingredients of *Kalka* added. Then thoroughly stirred while adding *Kwatha*. Then it will be heated.

- The temperature maintain will be in between 50ºC to 90ºC during the first hour of heating. Heating will be continuing for three hours and then stopped. Allow to stand overnight. Heating will be again started on next day while keeping a watch over the subsidence of froth (*Phena Shanti*) and the *Kalka* will be constantly checked for formation of *Varti (Madhyama Paka Lakshana).* Heating will be stop when *Varti* will be formed and froth subside.

- *Ghrita* was filtered while still hot (approx. 80º) through a muslin cloth and allowed to cool. After that, the *Ghrita* will be pack tightly in glass containers to protect from light and moisture.

4. MICROBIOLOGICAL EVALUATION:[iv]

Microbiological investigation has been carried out of *Mahatriphaladi Ghrita* after 90 days from day of preparation at Microbiological laboratory of I.P.G.T and R.A., GAU, Jamnagar. Smear examination and Aerobic as well as fungal culture study has been carried out for *Mahatriphaladi Ghrita* under microscope.

Smear examination: Gram's Stain and 10% KOH Preparation of *Mahatriphaladi Ghrita* has been done. A sterile sample smear collected under aseptic condition.

Culture study: Aerobic and Fungal culture has been assessed for the sample of *Mahatriphaladi Ghrita*. Aerobic culture has been carried out after 48hours. of incubation at 37degree Celsius in aerobic atmosphere and fungal culture after 07 days of incubation at 37-degree Celsius in aerobic condition.

PHARMACOGNOSTICAL EVALUATION:[v]

Morphological, organoleptic and microscopic evaluation of raw drugs which are used in *Mahatriphaladi Ghrita* were conducted at Pharmacognostical laboratory of institute. The *Ghrita* dissolve in small quantity of distilled water and studied with and without staining. Micro photographs of the slides were taken with Carl Zeiss trinocular microscope attached with camera.

ORGANOLEPTIC STUDY:

Contents of *Mahatriphaladi Ghrita* was evaluated for organoleptic characters as below. (Table 1).

PHARMACEUTICAL EVALUATION:

A. Physico-chemical analysis:[vi]

Physico-chemical Parameters of *Mahatriphaladi Ghrita* like loss on drying, Specific gravity many more were determined as per the API guideline. *Mahatriphaladi Ghrita* was further subjected to High Performance Thin Layer Chromatography (HPTLC) study.

B. HPTLC method High Performance:[vii]

Thin layer chromatography (HPTLC) studies were carried out with acid hydrolysed methanolic extract on pre-coated silica gel GF 60254 aluminium plate as 5mm bands, 5mm apart and 1cm from the edge of the plates, by means of a Camag Linomate V sample applicator fitted with a 100 µL Hamilton syringe. The mobile phase used was Toluene: Ethyl acetate: Glacial acetic acid: Formic acid (5:5:1:0.5). The plates were developed in Camag twin trough chamber (20 x 10 cm2) and spots were detected in short U.V. (254 nm), Long U.V (366nm). Camag Scanner II (Ver. 3.14) and Cats software (Ver. 3.17) were used for documentation.

5. RESULTS AND DISCUSSION:

The *Mahatriphaladi Ghrita*, used in this study showed results which has been analysed for Microbiological, Pharmacognostical and analytical parameters, which is a step towards standardization of the drugs.

5.1. MICROBIOLOGICAL EVALUATION:

Microbiological study under microscopic examination shows absence of any microorganisms on gram's stain and no organisms isolated after 48 hours. of incubation at 37degree Celsius under Aerobic Atmosphere. Similarly, for fungal culture, reveals absence of fungal filaments in 10%K.O.H preparation and on culture No fungal pathogens found as shown in plate 3.

5.2. PHARMACOGNOSTICAL EVALUATION:[viii]

5.2.1. Microscopic study: Diagnostic microscopic characters of ingredients of *Mahatriphaladi Ghrita* showed the following: (Plate 1).

DRAVYA	FINDINGS
Haritaki.	scleroids, stone cells and Rosette crystals
Bibhitaki	Rosette crystal and brownish coloured matter and Pitted scleroids
Amalaki	Silica deposition and Groups of fibres
Bhringaraja	Watery trichomes with base and Pollen grain
Vasa	Multicellular trichome and Annular vessels
Shatavari,	Cork in surface and Acicular cells
Guduchi	Cork cells and stone cells
Neel Kamal	Simple trichome
Yashtimadhu	Rhomboidal crystal
Kshirakakoli substitute	prismatic crystals and epidermal cells
Draksha	Acicular crystals and lignified parenchymal cells
Gambhari	lignified cork
Kantakari	Stellate trichome
Pippali	Oil globule

5.2.2. Physicochemical tests:

Pharmaceutical analysis of *Mahatriphaladi Ghrita* showed that loss on drying 00.39%w/w, Acid value 3.038%, Iodine value 44.343 %, Saponification value 188.01%, Specific gravity 0.9126% at 40degree Celsius Refractive index 1.4%. (Table-2).

6. HPTLC STUDY RESULTS:

Chromatographic study (HPTLC) was carried out under 254 and 366 nm UV to establish fingerprinting profile of *Mahatriphaladi Ghrita* which showed 09 of spots at 254 nm and 06 spots at 366nm with Rf values were recorded which may be responsible for expression of its pharmacological and clinical actions. Table 3. (Plate 2.)

Table 1: Organoleptic characters of *Mahatriphaladi Ghrita.*

Various parameters of Mahatriphaladi Ghrita	
Colour	Yellow
Odour	Aromatic
Taste	Astringent (Kashaya), Bitter (Tikta)
Touch	Soft
Texture	Smooth

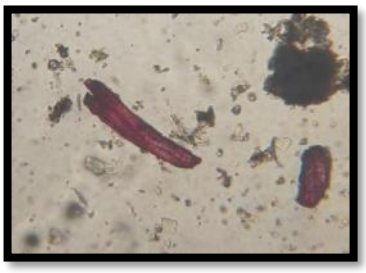
Table 2: Physico-chemical parameters of *Mahatriphaladi Ghrita*.

Analytical Parameter	Values
Loss on Drying	0.39%w/w
Acid value	3.038 N
Saponification value	188.01%
Specific gravity	0.9126 40deg. Celsius
Iodine value	44.343%
Refractive index	1.4240

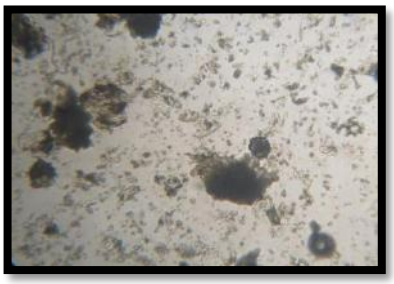
Table 3: High performance thin layer chromatography (HPTLC)

Sample	No. of spot	Observation	Max. R_f value
Mahatriphaladi Ghrita	**09**	Observed under short UV Light (254 nm)	44.6,22.1,89.8,48,186.7,44.8,57.1,111.7,117.4
Mahatriphaladi Ghrita	**06**	Observed under long UV Light (366 nm)	44.6,22.1,89.8,48,186.7,44.8,57.1

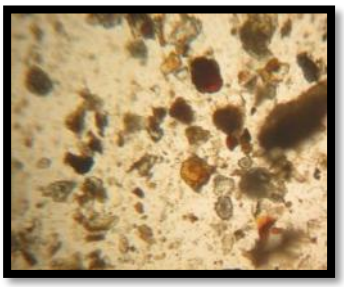
[Plate-1]



1.Sclereids and Stone cells of *Haritaki*

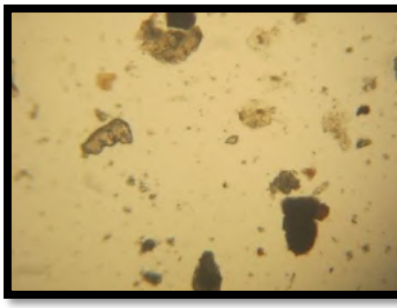


2.Rosette crystals of *Haritaki*

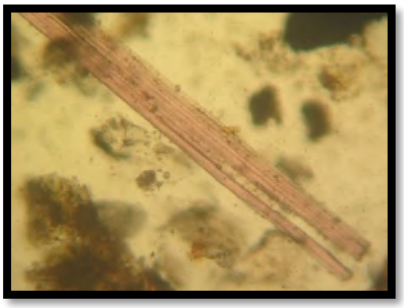


3.Rosette crystal brownish coloured matter of Bibhitakadi

4. Pitted scleroids of *Bibhitaki*

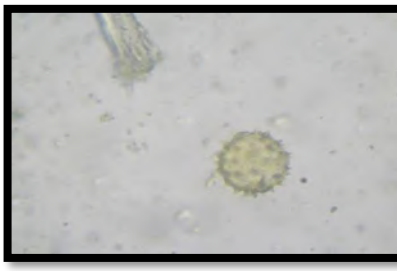


5.Sillica deposition of *Amalaki*

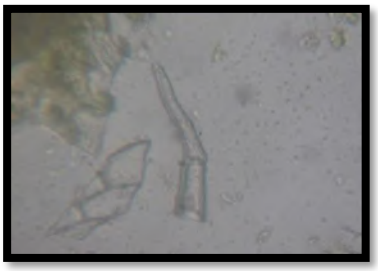


6.Groups of fibres of *Amalaki*

6.Watery trichomes of *Bhringaraja*

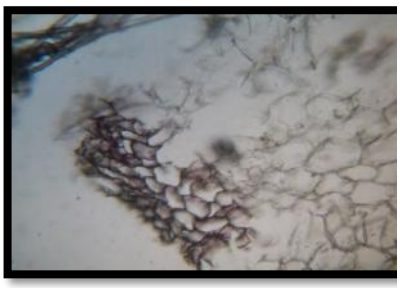


7.Pollen grain of *Bhringaraja*

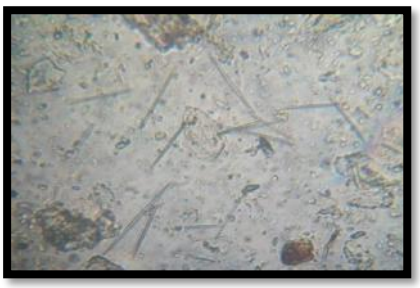


8.Multicellular trichome of *Vasa*

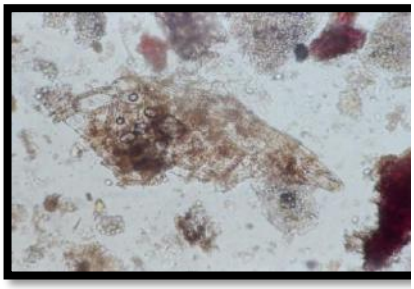
9.Anular vessels of *Vasa*



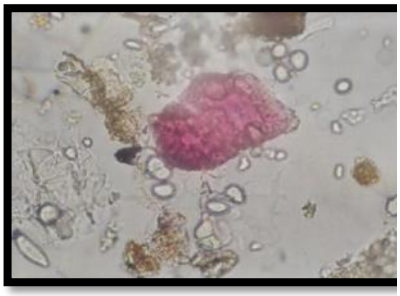
10.Corkin surface of *Shatavari*



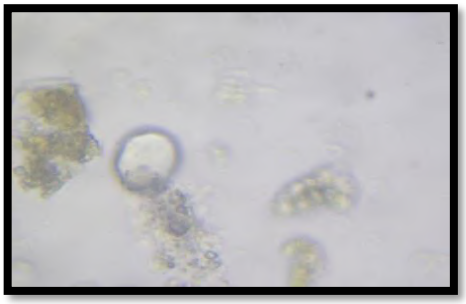
11.Acicular cells of *Shatavari*



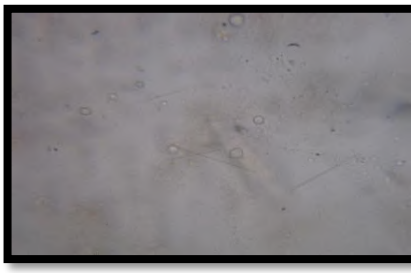
12.Cork cells of *Guduchi*



13.Stone cells of *Guduchi*

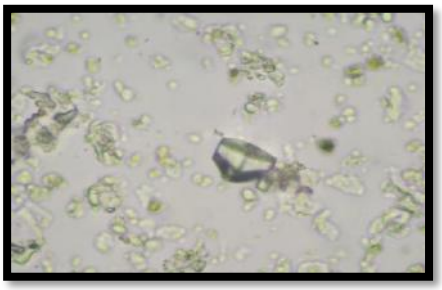


14.Oil globule of *Pippali*

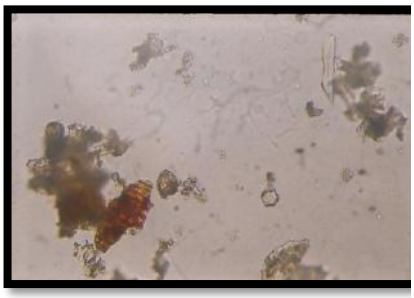


15.Acicular crystals of *Draksha*

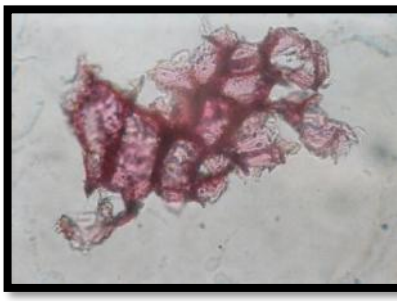
16.Simple trichome of *Neel Kamal*



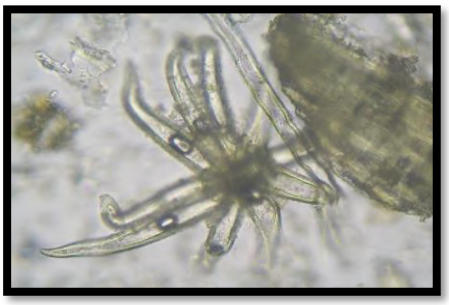
17.Rhomboidal crystal of *Yashtimadhu*



18.Prismatic crystals of *Kshirakakoli* substitute

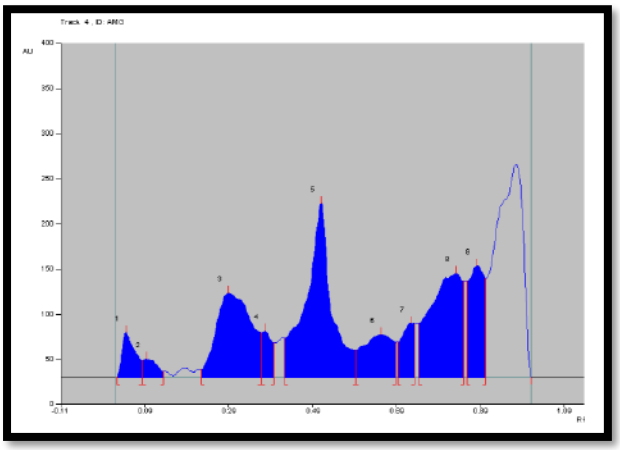


19.lignified cork of *Gambhari*

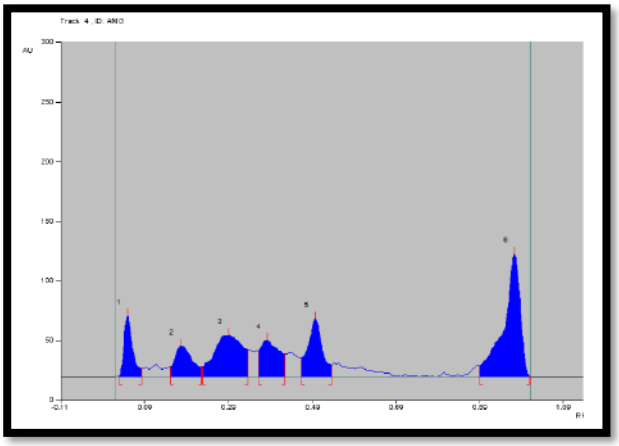


20.Stellate trichome of *Kantakari*

Plate-2

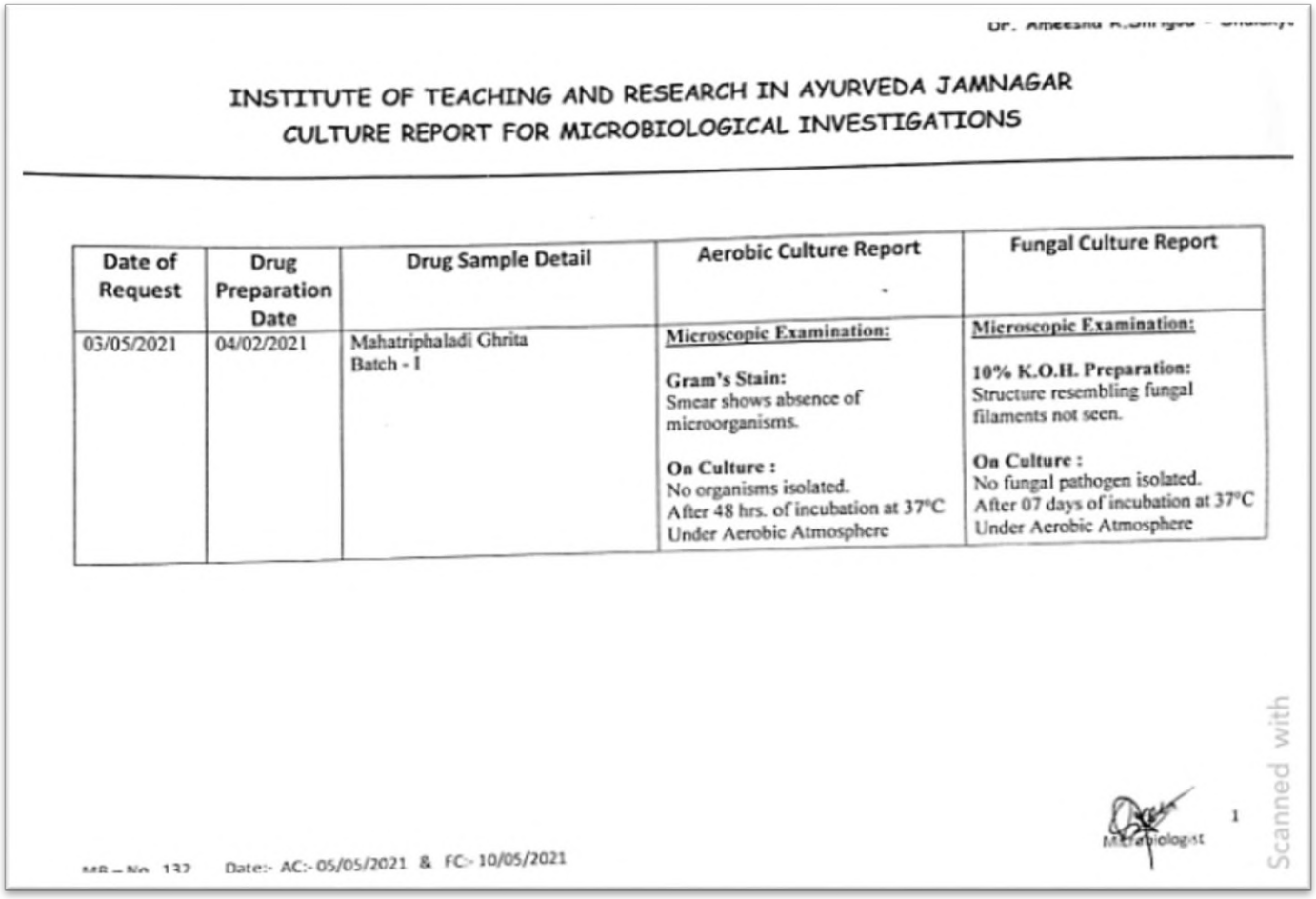


Observed under short UV light (254nm)
Mahatriphaladi Ghrita



Observed under long UV light (366nm)
Mahatriphaladi Ghrita

Plate-3



INSTITUTE OF TEACHING AND RESEARCH IN AYURVEDA JAMNAGAR
CULTURE REPORT FOR MICROBIOLOGICAL INVESTIGATIONS

Date of Request	Drug Preparation Date	Drug Sample Detail	Aerobic Culture Report	Fungal Culture Report
03/05/2021	04/02/2021	Mahatriphaladi Ghrita Batch - I	**Microscopic Examination:** **Gram's Stain:** Smear shows absence of microorganisms. **On Culture :** No organisms isolated. After 48 hrs. of incubation at 37°C Under Aerobic Atmosphere	**Microscopic Examination:** **10% K.O.H. Preparation:** Structure resembling fungal filaments not seen. **On Culture :** No fungal pathogen isolated. After 07 days of incubation at 37°C Under Aerobic Atmosphere

Microbiologist

MR – No. 132 Date:- AC:- 05/05/2021 & FC:- 10/05/2021

Scanned with

7. CONCLUSION:

Microbiological, Pharmacognosy and phytochemical evaluation of *Mahatriphaladi Ghrita* was performed. Microbial study assessed by two different methods shows no microbial contamination in the sample of *Mahatriphaladi Ghrita*. Preliminary Organoleptic features and results of powder microscopy shows the ingredients which were used confirm the gentility and quality of *Mahatriphaladi Ghrita*. All the ingredients were proved to be authentic and compared with the parameters mentioned in API (Ayurvedic Pharmacopeia of India). Pharmaceutical

Evaluation shows values which fits for quality of *Ghrita* originality. Though the groundwork requisites for the standardization of *Mahatriphaladi Ghrita* are covered in the current study, additional important analysis investigations are required for the identification of all the active chemical constituents of the test drug to substantiate the clinical efficacy.

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