JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"STABILITY STUDY OF *PANCHAGAVYA GHRITA*, WITH RESPECT TO BASELINE MICROBIAL DIAGNOSTIC MODALITIES."

Sudesh Pardhi[*1], Dr. M. S. Cholera[2], Dr. A.G. Vyas [3]

1. PG scholar, Department of Kaumarabhritya, ITRA Jamnagar.
2. Head Microbiology Laboratory, ITRA Jamnagar.
3. Reader, Department of Kaumarabhritya, ITRA Jamnagar.

ABSTARCT

Background: *Panchagaya Ghrita* is an Ayurvedic Formulation mentioned in *Asthanga Hriday* in *Uttarsthana* in *Apasmara Patishedha adhyaya*. It contains 5 ingredients which are *Godugdha* (cow's milk), *Godadhi* (cow's curd), *Gomutra* (cow's urine), *Goshakrita* rasa (cow dung juice), and *Go-ghrita* (cow's ghee). PG is indicated in medical conditions like *Apasmara* (epilepsy), *Jwara* (pyrexia), and *Kamala* (Jaundice). PG is widely used nowadays for the management of Neurobehavioral disorders and Neurodevelopment disorders like ADHD, Autism, Cerebral Palsy, etc. Due to its wide spectrum of use in different diseases this ayurvedic formulation is widely spread in the Ayush doctors and is used globally. As the PG, lacking in its data of *Saviryatavadhi* (shelf-life period), the present study was carried out to evaluate the stability of *Panchagavya Ghrita* with respect to microbial contamination and fungal growth.

Aim: To check the stability and to check microbial contamination in the finished product at different time interval- at different climatic conditions, temperature and humidity set ups. **Materials & Methods:** In present study, stability with respect to its Microbial profile of *Panchagavya Ghrita* was carried out. It was stored in Plastic container during different climacteric conditions were studied at regular intervals for a period of eight months to analysis presence no microbial growth by Smear and culture study respectively. At the end of study *Panchagavya Ghrita* had no presence of microbes or bacteria throughout the study 8 months from the day sample preparation even in different climate and temperature. **Results:** In present study, the stability test of *Panchagavya Ghrita* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions. **Conclusion:** Results of the present study can be used as quality parameters for *Panchagavya Ghrita* and hence will be helpful as reference guidance for future scientific evaluations.

KEY WORDS: Ayurveda, Stability, Microbial profile, *Panchagavya Ghrita*.

Introduction: In the *Snehakalpanas*, *Ghrita* is considered as the best one[1]. *Ghrita* is *Yogavahi* which not only incorporate additional pharmacological qualities of the host but also doesn't lose its original properties. *Panchagaya Ghrita* is an Ayurvedic Formulation which medicinal benefits are innumerable. *Panchagavya* is derived from two-word "*Pancha*" means

five and "*Gavya*" means substances which are derieved from cow. It contains 5 ingredients which are *Godugdha* (cow's milk), *Godadhi* (cow's curd), *Gomutra* (cow's urine), *Goshakrita* rasa (cow dung juice), and *Go-ghrita* (cow's ghee). *Asthangahridaya* mentioned *Panchagavya ghrita* having the properties of promoting intelligence, memory instantaneously and by using this *Ghrita* we can treat diseases like *Apasmara*, *Jwara*, *Unmada*, *Kamala*[2]. Due to its wide spectrum of use in different diseases this ayurvedic formulation is widely spread in the Ayush doctors and is used globally. As the PG, lacking in its data of *Saviryatavadhi* (shelf-life period), the present study was carried out to evaluate the stability of *Panchagavya Ghrita* with respect to microbial contamination and fungal growth.

No any preservative was added to the test drug. Drug preparation was finished on 23/06/2023. Finished product was stored in airtight plastic containers at room temprature. It was necessary to prepare the formulation in a better form which is also free from microbial contamination, stability of a pharmaceutical product is the capability of a perticular formulation in a specific container or closure system, to remail within its physical, chemical, microbiological therapeutic specifications. Thus in the present study on attempt was taken to check stability of Ghrita with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 10 Months 7 days.

AIM: To study the stability of finished product and to check microbial contamination in the finished product at different time interval- at different climatic conditions, temperature and humidity set ups.

Materials and Methods:

Sample of *Panchagavya Ghrita* was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals for a period of 10 Months 7 days (upto drug used). Microbiological study has been carried out in Microbiology Laboratory, ITRA, Jamnagar. Mainly 02 studies have been carried out to rule out that presence of any bacteria or fungi in the prepared drug as a final finished product.

The initial microbiological study was done on 8th day of preperation, before giving it to the patients. Then samples from same container were subjected to the microbilogical study regularly with random intervals during different seasons.

Drug material:

All the Ingredients of *Panchagavya ghrita* were procured from the outside, Authenticated for quality and purity by the experts of Pharmacognosy laboratory, ITRA, Jamnagar. The ingredients are given in **(Table 1)**.

Table 1: Ingredients of *Panchagavya Ghrita* [3]

S. no.	Ingredients	English name	Proportion
1.	*Go-Ghrita*	Cow Ghrit	1 part
2.	*Go-Dugdha*	Cow milk	1 part
3.	*Go-Dadhi*	Curd of cow	1 part
4.	*Go-Mutra*	Cow's Urine	1 part
5.	*Go-shakrita rasa*	Juice of Cow dung	1/4th part

Date of Drug Preparation: 23rd June 2023

Storage:

Finished product of *Panchagavya Ghrita* was stored in air-tight food grade, plastic containers, stored in the open light area in the department at room temperature. Clean and dry stainless steel spoon was used to take medicine.

MICROBIAL PROFILE:

To check any mycological findings and bacteriological findings, microbial contamination was estimated by Different modalities.

Table 2: Methods for estimation of microbial contamination

1. Smear Examination	-Wet mount /10% K.O.H. Preparation -Gram's stain

A. SMEAR EXAMINATION: -

1. PROCEDURE FOR 10% KOH PREPARATION:

Take Potassium Hydroxides pellets (of HiMedia Lab. Pvt. Ltd.) in distilled water to prepare 10% of the same in clean glass tube & mix well

⇩

Take clean grease free glass slide

⇩

Put a-drop of specimen and add freshly prepared 10% KOH then cover it with grease free cover glass

⇩

Allow it to react for 15-20 minutes to remove extra debris other than fungal particles

⇩

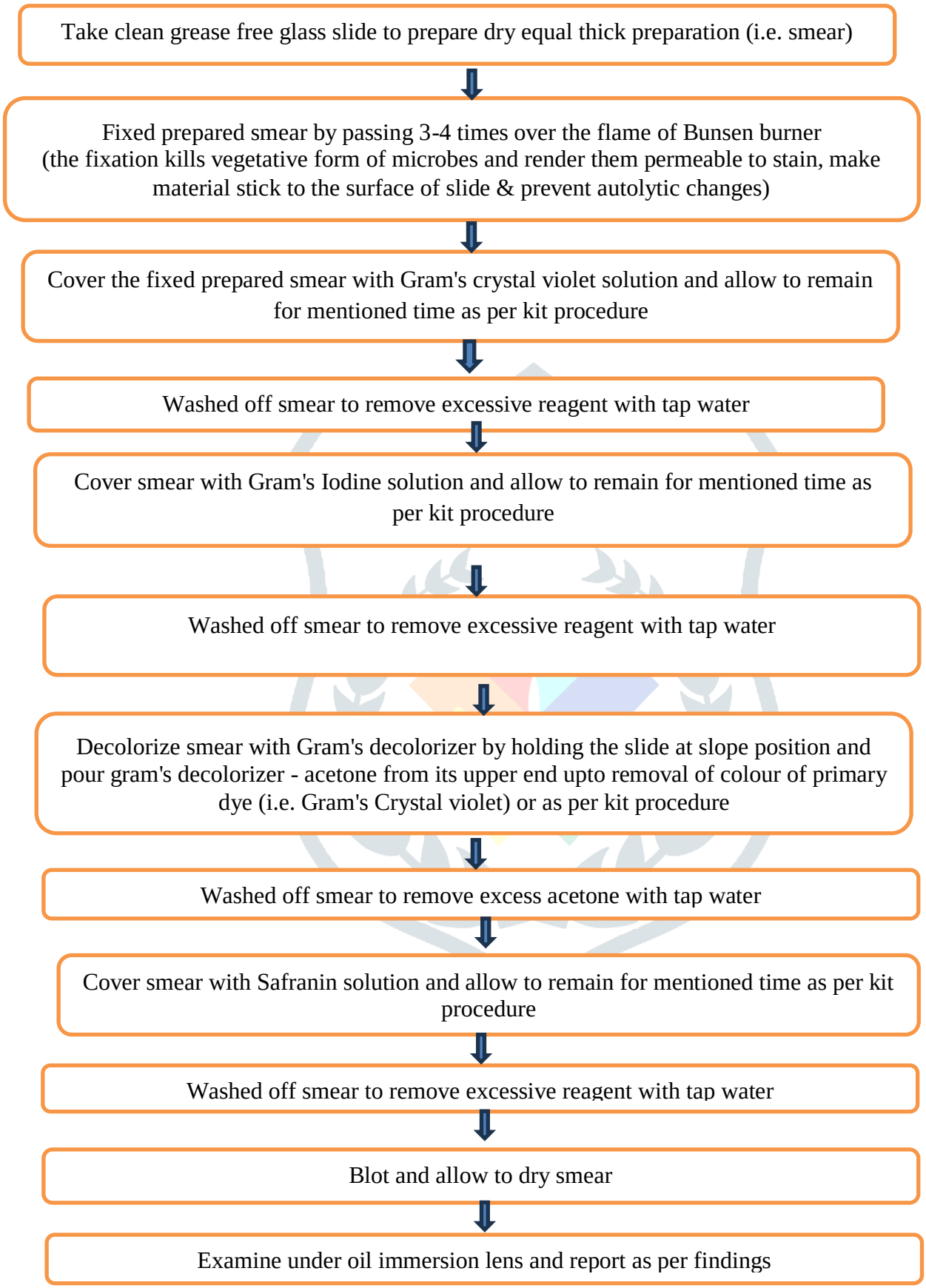
Observe under high power (40x) microscopic lens

⇩

Report as per finding

.

2. PROCEDURE FOR GRAM'S STAIN TEST:



B. CULTURE STUDY: -

1. FUNGAL CULTURE METHOD:

Respected materials collected with sterile cotton swab for inoculation purpose on selected fungal culture media (i.e. an artificial preparation).
Name of media: Sabouraud Dextrose Agar Base (SDA), Modified (Dextrose Agar Base, Emmons)
Company: HIMEDIA Laboratories Pvt. Ltd.
Required time duration: 05 to 07 days
Required temperature: 37 ºC
Use of media: For selective cultivation of pathogenic fungi.

Figure 1. Sabouraud Dextrose Agar Base (SDA) bottle

Procedure for Fungal Culture:

In the clinical microbiology laboratory culture method are employed for isolation of organisms (The lawn / streak culture method is routinely employed)

⇩

Choose appropriate selective solid media for inoculation purpose

⇩

Dry selective solid media in Hot Air Oven before specimen inoculation, allow to cool dried medium before specimen inoculation

⇩

Inoculate selected *Panchagavya Ghrita* by Sterile cotton swab or by Nichrome wire (24 5.W.G. size) loop [First sterile loop in Bunsen burner oxidase flame blue flame and allow it to cool than loop is charged with selected specimen to be cultured. One loopful of the specimen is transferred onto the surface of well dried culture media]

⇩

After inoculation / streaking process Incubate inoculated medium in inverted position at 37°C for 05 to 07 to 21-days in Incubator (incubation days are as per growth requirement) under aerobic atmosphere

⇩

After selected incubation period examined growth by naked eye in form of colony or arial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates.

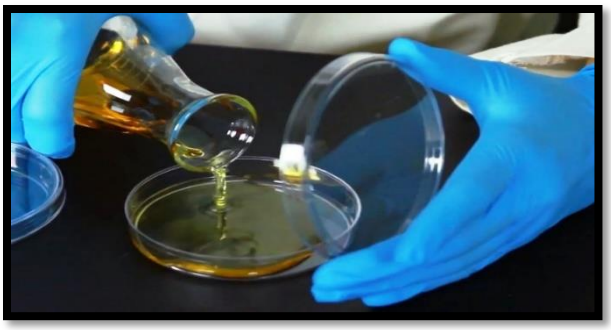


Figure 2: Procedure for Fungal culture

2. AEROBIC CULTURE METHOD:

Respected materials collected with sterile cotton swab for inoculation purpose on selected fungal culture media (i.e. an artificial preparation).

Name of media: MacConkey Agar (MA) and Columbia Blood agar (BA)
Company: HIMEDIA Laboratories Pvt. Ltd.
Required time duration: 24 to 48 hours
Required temperature: 37 ºC
Use of media: For selective cultivation of pathogenic bacteria.

Figure 3: Mac Conkey Agar (MA)

Procedure for Aerobic Culture: -

In the clinical microbiology laboratory culture method are employed for isolation of organisms (The streak culture method is routinely employed)

⇩

Choose appropriate selective solid media for inoculation purpose

⇩

Dry selective solid media in Hot Air Oven before specimen inoculation, allow to cool dried medium before specimen inoculation

⇩

Inoculate selected *Panchagavya Ghrita* by four flame method (the loop should be flamed and cooled between the different sets of streaks i.e. four time) on surface of cool dried medium with Nichrome wire (24 S.W.G. size) loop [First sterile loop in Bunsen burner oxidase flame-blue flame and allow it to cool than loop is charged with selected specimen to be culture. One loopful of the specimen was transferred onto the surface of well dried plate]

⇩

After streaking process Incubate inoculated medium in inverted position at 37°C for 18-24 hours in Incubator under aerobic or 10% CO2 atmosphere.

⇩

After selected incubation period examined growth by naked eye in form of colony and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates

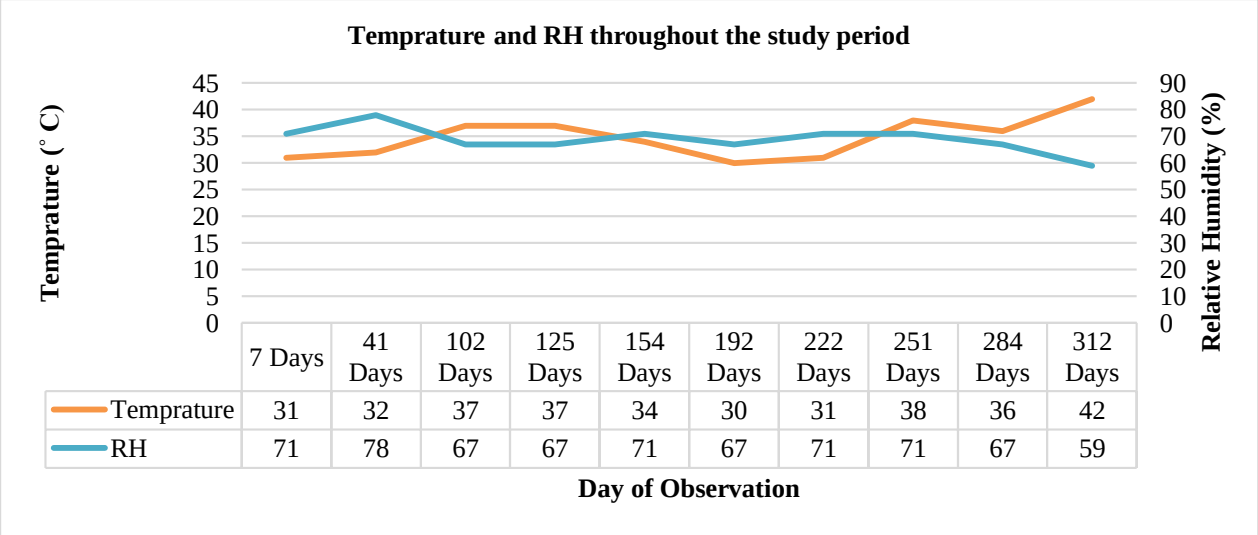
Figure 4: Procedure for Aerobic culture

Every time sample (in which drug preserved) were subjected to the microbiological study from the date of the preperation to the date of last microbiological study.

Table no. 3: Showing observations of sample of *Panchagavya Ghrita* preserved in air tight container (Preparation Date- 23/06/2023)

Sr. No.	Days of investigations After preparation of the sample	Humidity (%)	Temp (° C)	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	30/06/2023 7 days	71%	31° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
2.	3/8/2023 41 days	78%	32° C	Absence of Microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
3.	3/10/23 102 days	67%	37° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
4.	26/10/23 125 days	67%	37° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
5.	24/11/23 154 days	71%	34° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
6.	1/01/24 192 days	67%	30° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
7.	31/01/24 222 days	71%	31° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
8.	29/2/24 251 days	71%	38° C	Absence of Microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated

9.	2/4/24 284 days	67%	36° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
10.	30/4/24 312 days	59%	42° C	Absence of Microorganisms	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated



	7 Days	41 Days	102 Days	125 Days	154 Days	192 Days	222 Days	251 Days	284 Days	312 Days
Temprature	31	32	37	37	34	30	31	38	36	42
RH	71	78	67	67	71	67	71	71	67	59

Discussion: The present study was carried out to observe the stability study of *Panchagavya Ghrita* with respect to Microbial contamination of sample prepared and preserved in different climatic and temperature conditions as mentioned in table no 3. By observing table no 3 we can say that there is no any microbial growth found between minimum 30°C to maximum 42°C temperatures at minimum 59% of relative humidity and maximum 78% relative humidity.

No any microbes were isolated as a result of aerobic culture and no any fungal pathogens were isolated as a result of fungal culture of *Panchagavya Ghrita* sample during this study period.

Conclusion: Shelf- life is the time period from when the product is produced until the time it is planned to be consumed or used. Several factors are used to determine a product's shelf-life, ranging from organoleptic qualities to microbiological safety. Hence Microbiological study of the *Panchagavya Ghrita* showed that the quality of *Ghrita* is in a standard condition. There were no growth found of microorganisms (bacterial or fungal), till 30th April 2024 i.e. 10 months 4 days from the date of preparation, shows its good shelf life.

FINANCIAL SUPPORT:
The study was supported by the Institute of Teaching and Research (ITRA), Jamnagar, Gujarat, India.

CONFLICTS OF INTEREST:
There are no conflicts of interest.

REFRENCES

[1] Vaghbhata's Astangahrdayam. translated by Srikanta Murthy K.R. vol 1Ed Varanasi: Chaukambha Krshnadas Academy: 2009 16/ p.208

[2] Vaghbhata's Astangahrdayam. translated by Srikanta Murthy K.R.vol-3 Ed Varanasi: Chaukambha Krishnadas Academy: 2009 7/70

[3] Acharya Jadavji Trikamji, editor. Charak Samhita of Agnivesha with Ayurveda Dipika commentary of Chakrapanidatta, Chikitsa Sthana. Ch. 10, Ver.17, Reprint edition. Varanasi:Chaukhambha Prakashan: 2011/ p.475