"STABILITY STUDY OF *HARITAKI SHUNTHI CHURNA,* USED IN TREATMENT OF *AJIRNA* (INDIGESTION)- WITH RESPECT TO BASELINE MICROBIAL DIAGNOSTIC MODALITIES."

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ABSTARCT:

Ajirna is the most common complaint in day to day life. *Agnimandya* is the main cause of indigestion. Indigestion can be caused by both *Ahara* and *Aushadh,* of which indigestion of *Aushadh* is more harmful. Prior to manifestation of any disease *Ajirna* is happened. That why *Ajirna* is said as a root cause of all the disease. Aacharyas have mentioned various *Shamana* and *Shodhana* procedures which are useful in the treatment of *Ajirna*. In present study, *Haritaki Sunthi Churna* for treatment of *Agnimandhya* has mentioned in Bhavaprakash was used for treatment of *Ajirna*. In present study, stability with respect to its Microbial profile of *Haritaki Sunthi Churna* was carried out. Stability means the ability of the pharmaceutical dosage form to maintain the physical, chemical, therapeutic and microbial properties during the time of storage and usage by patients. Hence the present Study was carried out to observe the stability study of *Haritaki Sunthi Churna* with respect to Microbial Contamination of sample prepared and preserved in minimum 33% & maximum 89% of Humidity and minimum 33^0 C & Maximum 40^0 C temperature. Thus a baseline Microbial profile was studied at regular time interval Up to 250^{th} day from the date of drug preparation. At the end of study it was found that sample was not showed presence of any Microbes.

KEY WORDS: Stability, Microbial profile, *Haritaki Shunthi Churna*, Climate conditions.

Introduction:

Ajirna is the most common complaint in day to day life. Acharya Susruta has 4 types of *Ajirna* with its treatment i.e. *Amajirna – Langhan, Vidagdhajirna – Vamana, Vishtabdhajirna – Swedana, Rasasheshajirna – Shayana.*[1] Treatment is depend upon *Agni*, in *Ajirna* vitiation of *Agni* is the main cause. Due to vitiation of *Agni*, prior to manifestation of any disease *Ajirna* is happened. That why *Ajirna* is said as a root cause of all the disease. *Agnimandya* is the main cause of indigestion.

In this context Aacharya Bhavprakasa described *Haritaki Shunthi Churna* in *Ajirna or Agnimandhya.* Aacharya Bhavprakasa also Further explain that *Haritaki Shunthi Churna* is having *Katu Rasa, Ushna Veerya* and *Madhura Vipaka* is best for improving strength of *Jathargni* when consumed with *Saindhava* or *Guda*.

The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar. No any preservative was added to the test drug. Drug preparation was finished on 13/02/2019. Finished product was stored in airtight plastic containers at room temperature. 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 particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological therapeutic specifications.

Hence the present Study was carried out to observe the stability study of *Haritaki Sunthi Churna* with respect to Microbial Contamination of sample prepared and preserved in minimum 33% & maximum 89% of Humidity and minimum 33^0 C & Maximum 40^0 C temperature. Thus a baseline Microbial profile was studied at regular time interval Up to 250^{th} day from the date of drug preparation.

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

Materials and Methods:

- Sample of *Haritaki- Sunthi Churna* was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular time intervals up to 250^{th} day (21/10/2019) from the date of drug preparation (13/02/2019).
- Microbiological study has been carried out in Microbiology Laboratory, I. P. G. T. & R. A., 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 7th day of preparation, before giving to the patients. Then samples from same container were subjected to the microbiological study regularly with random intervals during different seasons.

Drug material:

All the raw drugs were obtained from Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients and the part used are given in (Table 1).

Table 1: Ingredients of *Haritaki- Sunthi Churna*[2]*:*

No.	Name of Drug	Botanical name	Part used	Proportion
1.	*Haritaki*	*Terminalia chebula* Retz.	Fruit pericarp	1 part
2.	*Sunthi*	*Zingiber officinale* Roscoe.	Rhizome	1 part

DRUG PREPARATION DATE: 13/02/2019

STORAGE: - Drug was stored in plastic containers at room temperature in a cool, dark and dry place. Samples were subjected to stability study with respect to microbial and fungal contamination at regular time intervals. Details of which are cited below.

MICROBIAL PROFILE: Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.

1. **Smear Examination-**
    A) Wet mount /10% KOH. Preparation
    B) Gram's stain
2. **Culture Study-**
    A) Fungal culture
    B) Aerobic culture

The details of the procedures followed are given below.

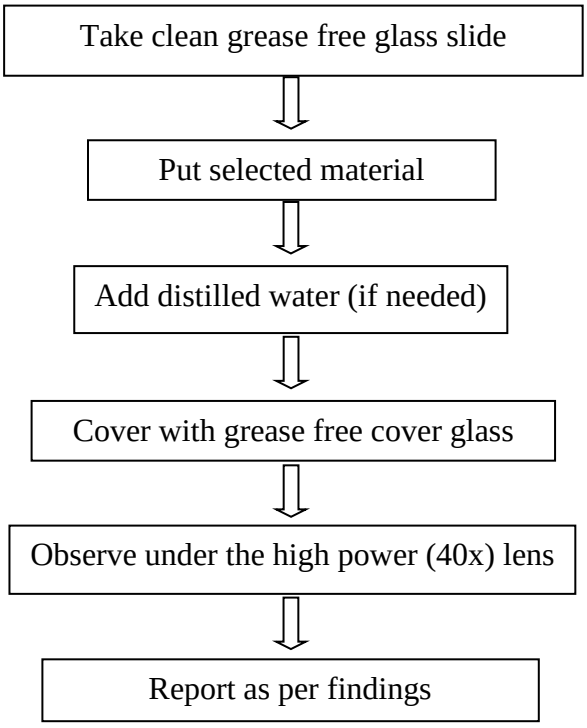
1. Smear Examination:

A. Wet mount /10% KOH. Preparation:

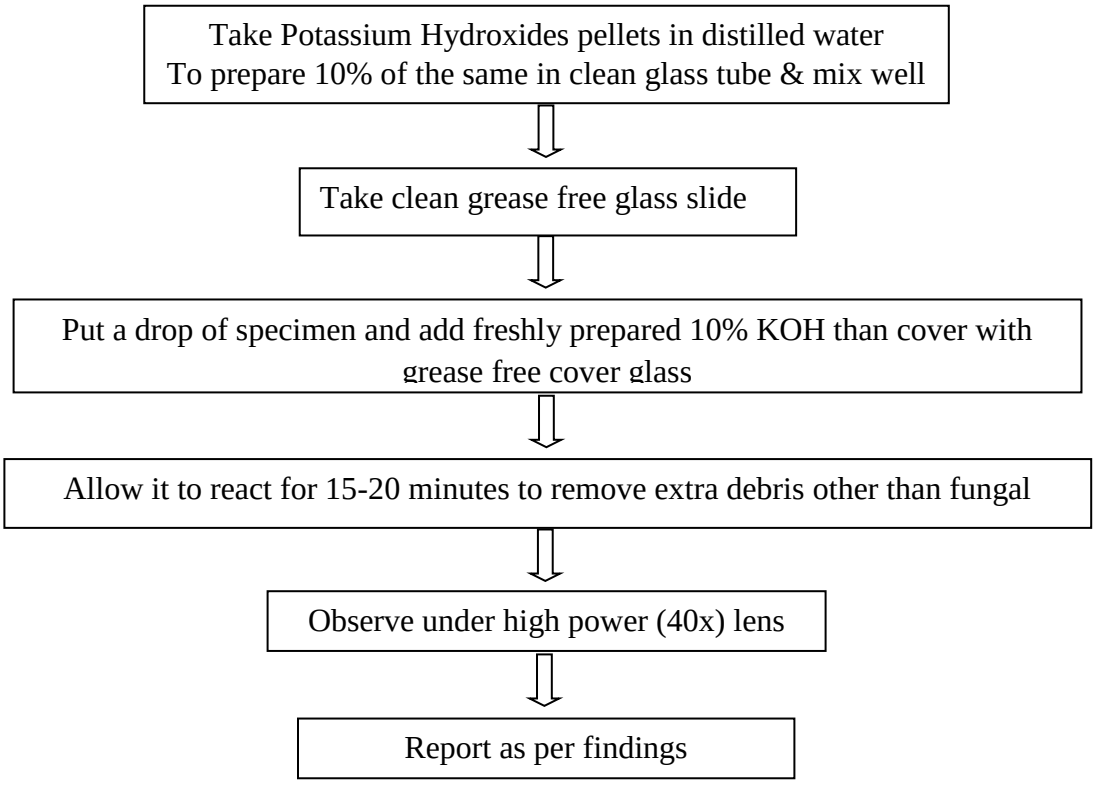
Aim: To rule out any mycological findings.

Specimen: *Haritaki- Sunthi Churna*

PROCEDURE FOR WET PREPARATION



Procedure For 10% KOH Preparation



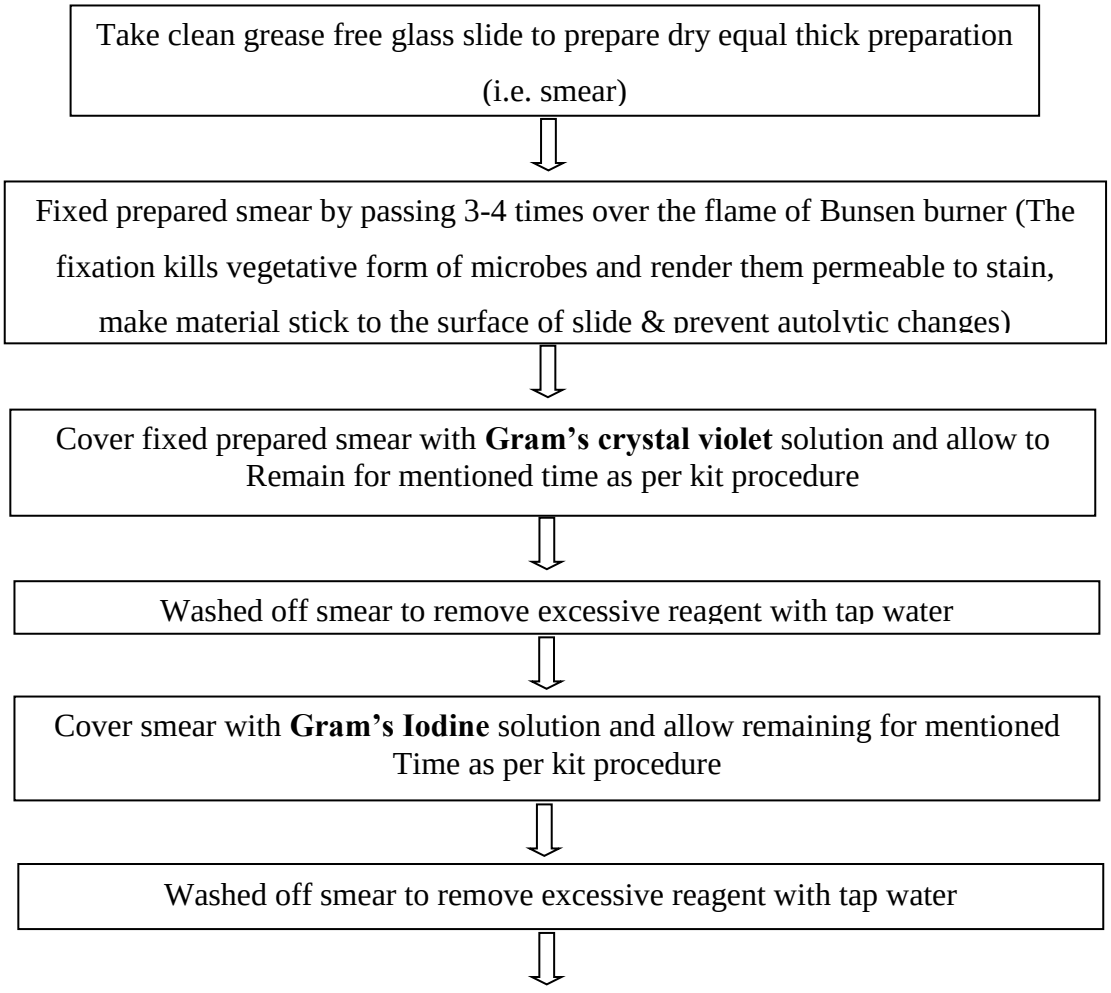
B. Gram's stain test:-

Gram staining is a differential staining technique that differentiates bacteria into two groups: Gram positive and Gram negative. The procedure is based on the ability of microorganisms to retain colour of the stains used during the gram stain procedure. Gram negative bacteria are decolorized by any organic solvent (acetone or Gram's decolorizer) while Gram positive bacteria are not decolorized as primary dye retained by the cell and bacteria will remain as purple. After decolonization step, a counter stain effect found on Gram negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain color of the stains, based on the differences in the chemical and physical properties of the cell wall. (Alfred E Brown, 2001)[2]

AIM: To rule out any bacteriological findings.

Specimen: *Haritaki- Sunthi Churna*

Procedure for Gram's Stain



Decolourize smear with **Gram's decolourizer** by holding the slide at slope position and pour gram's decolourizer – acetone from its upper end upto removal of colour of primary dye (i.e. Gram's Crystal Violet) or as per kit procedure

⇩

Washed off smear to remove excessive reagent with tap water

⇩

Cover smear with **Safranin** solution and allow remaining for mentioned Time as per kit procedure

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Washed off smear to remove excessive reagent with tap water

⇩

Blot and allow to dry smear

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Examine under oil immersion lens and report as per finding

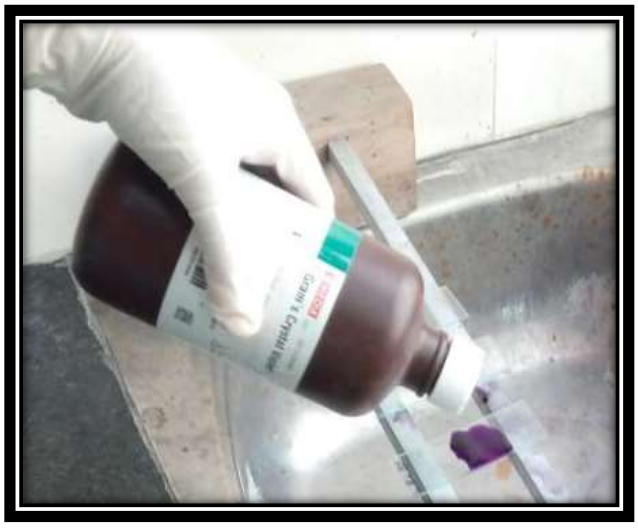
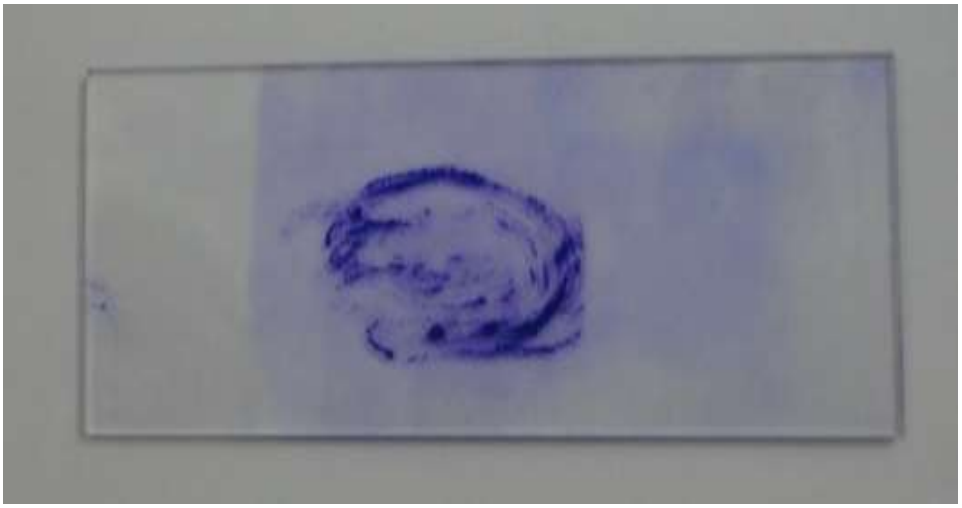


Figure 01. & 02. Smear staining Procedure



1. Culture Study

A. 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 03. 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)

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

⇩

After inoculation / streaking process incubate inoculated medium in inverted position at 37^0 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 necked 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.

B. **Aerobic culture method:**

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

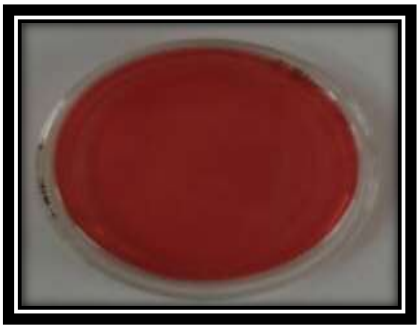
Name of media : Mac Conkey Agar (MA) and Columbia Blood agar (BA)

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.



Observation & Result: The initial microbiological study of *Haritaki Shunti Churna* was done on 7th day of preparation, before giving to the patients. Then further study was carried out at regular time interval up to 250th day. Observation and result of the study with humidity and temperature are depicted in table no.2.

Table 02: Results microbiological study of *Haritaki- Sunthi Churna* (Drug preparation date:- 13/02/2019)

Sr. No.	Days of investi-gation after prepar-ation drug	Date of Sample given Temp. & Humidity	Observations / Findings			
			Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	7th day	20/02/19 36^0 C, 33%	Microorganisms Not Seen	No organisms isolated in drug	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	34th day	19/03/19 37^0 C, 39%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	91th day	15/05/19 40^0 C, 44%	Microorganisms Not Seen	No organisms isolated in drug	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	127th day	20/06/19 38^0 C, 55%	Microorganisms Not Seen	No organisms isolated in drug	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	152th day	15/07/19 36^0 C,	Microorganisms Not Seen	No organisms isolated in drug	Fungal filaments not seen.	No Fungal Pathogen Isolated

		72%				
6.	189th day	21/08/19 33^0 C, 89%	Microorganisms Not Seen	No organisms isolated in drug	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	215th day	16/09/19 33^0 C, 55%	Microorganisms Not Seen	No organisms isolated in drug	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	250th day	21/10/19 34^0 C, 58%	Microorganisms Not Seen	No organisms isolated in drug	Fungal filaments not seen.	No Fungal Pathogen Isolated

Discussion:-

Ayurveda is widely used in chronic disorders like *Ajirna* (indigestion). In present study *Haritaki Sunthi Churna* has given in 30 patients of *Ajirna* at I.P.G.T. & R.A. hospital, Jamanagar. It has shown very good result in symptoms of *Ajirna* and also improvement in *Abhyavaranshakti* and *Jaranashakti* is observed.

Shelf-life of a drug product is defined as the time at which the average drug characteristic (e.g. Potency) remains within an approved specification after manufacture (FDA, 1987). Drug stability means the ability of the pharmaceutical dosage form to maintain the physical, chemical, therapeutic and microbial properties during the time of storage and usage by patients. Common factors that affect this stability include temperature, light, humidity, pH, oxidation and enzymatic degradation. Stability is an essential quality attribute for pharmaceutical formulations. Evaluation of drug stability can prevent toxicity and increase safety, efficacy and quality of the final drug product.[3]

Hence the present Study was carried out to observe the stability study of *Haritaki Sunthi Churna* with respect to Microbial Contamination of sample prepared and preserved in minimum 33% & maximum 89% of Humidity and minimum 33^0 C & Maximum 40^0 C temperature. Thus a baseline Microbial profile was studied at regular time interval Up to 250th day from the date of

drug preparation. At the end of study it was found that sample was not showed presence of any Microbes.

Conclusion: Stability means the ability of the pharmaceutical dosage form to maintain the physical, chemical, therapeutic and microbial properties during the time of storage and usage by patients. Several factors are used to determine a product's shelf-life, ranging from organoleptic qualities to microbiological safety. Hence Microbiological study of the *Haritaki Sunthi Churna* showed that the quality of *Churna* is in a standard condition. There were no growth found of microorganisms (bacterial or fungal), 21/10/19 i.e. Up to 250th day from the date of drug preparation, shows its good shelf life.

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