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Exploration of stability of Abha Guggulu in the treatment of Knee Osteoarthritis, in alignment with Baseline Microbial Diagnostic Technique

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ABSTARCT: Aging is an inevitable process. As the age progresses person will be affiliated with many diseases. Among such Knee osteoarthritis is the most common degenerative disorder that affects the person's life significantly also in quality of Life. Owing to the side effects from the use of contemporary medicines, there is a need to the use of herbal drugs for the better outcome. Here Abha Guggulu was used for internal administration. In present study, stability with respect to its Microbial profile of Abha Guggulu was carried out. Drug was stored in plastic container during different climacteric conditions and were studied at regular intervals for a period of 7 months to analysis Mycological findings and presence of bacteriological findings by Wet mount preparation and Gram stain test respectively. At the end of study drug didn't show any presence of microbesafter7 months of preparation of sample, even in different climate and temperature. Hence in present study, the stability test of above-mentioned drug with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEY WORDS: ,Abha Guggulu, Stability, Microbial profile, Climate conditions.

I. INTRODUCTION

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 remail within its physical ,chemical, microbiological therapeutic specifications. Thus in the present study on attempt was taken to check stability of drug with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 7 months.

Osteoarthritis (OA) is a chronic degenerative disorder of multifactorial etiology characterized by the loss of articular cartilage, hypertrophy of bone at the margins, subchondral sclerosis and range of biochemical and morphological alterations of the synovial membrane and joint capsule[1]. It is a leading cause of chronic disability in the developed and developing countries. Osteoarthritis of Knee is extremely common by the age of 60. Pathologic changes in weight bearing joint can be seen in majority of the geriatric population. It is second most common Rheumatologic problem which is more common in women than men. The prevalence of OA of Knee in India is estimated to be 28.7%[2]Globally. In Ayurveda, Sandhigatavata is one among vatavyadhi and the line of treatment will be similar to Vata Vyadhi Chikitsa i.e., Swedana, Snehana, Lepa, Upanaha, Agnikarma.[3]In the present study, the patients were intervened with Abha Guggulu and its authentication and microbial profile carried out systematically by adopting standard operative procedure for churna preparation. No any preservative was added to the test drug. Drug preparation was finished on 26/09/2023.Finished products were stored in airtight plastic containers at room temprature.

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

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II. MATERIALS AND METHODS:

Sample of Abha Guggulu was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals for a period of 7 months (upto drug used). Microbiological study has been carried out in Microbiology Laboratory, I. 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 14th day of preperation, Before administering 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 raw drugs were obtained from Pharmacy of ITRA, Jamnagar. The ingredients and the part used are given in **(Table 1)**.

Table 1: Ingredients of Abha Guggulu (Chakradutta, Bhagnarogadhikara)

Sl. No.	Contents	Botanical name	Part used	No. of parts
1	Babbula	Acacia arabica	Stem Bark	1 Part
2	Guggulu	Commiphora mukul	Resin	7 Parts
3	Haritaki	Terminalia chebula	Fruit	1 Part
4	Vibhitaki	Terminalia bellirica	Fruit	1 Part
5	Amalaki	Emblica officinalis Linn	Fruit	1 Part
6	Shunti	Zingiber officinale	Tuber	1 Part
7	Maricha	Piper nigrum Linn	Fruit	1 Part
8	Pippali	Piper longum Linn	Fruit	1 Part

Date of Drug Preparation: 26th September, 2023
Storage:
Finished product of Abha Guggulu 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:
Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.

1. Smear Examination-
A) 10% K.O.H. 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. 10% K.O.H. Preparation:
Aim: To rule out any mycological findings.
Specimen: Abha Guggulu

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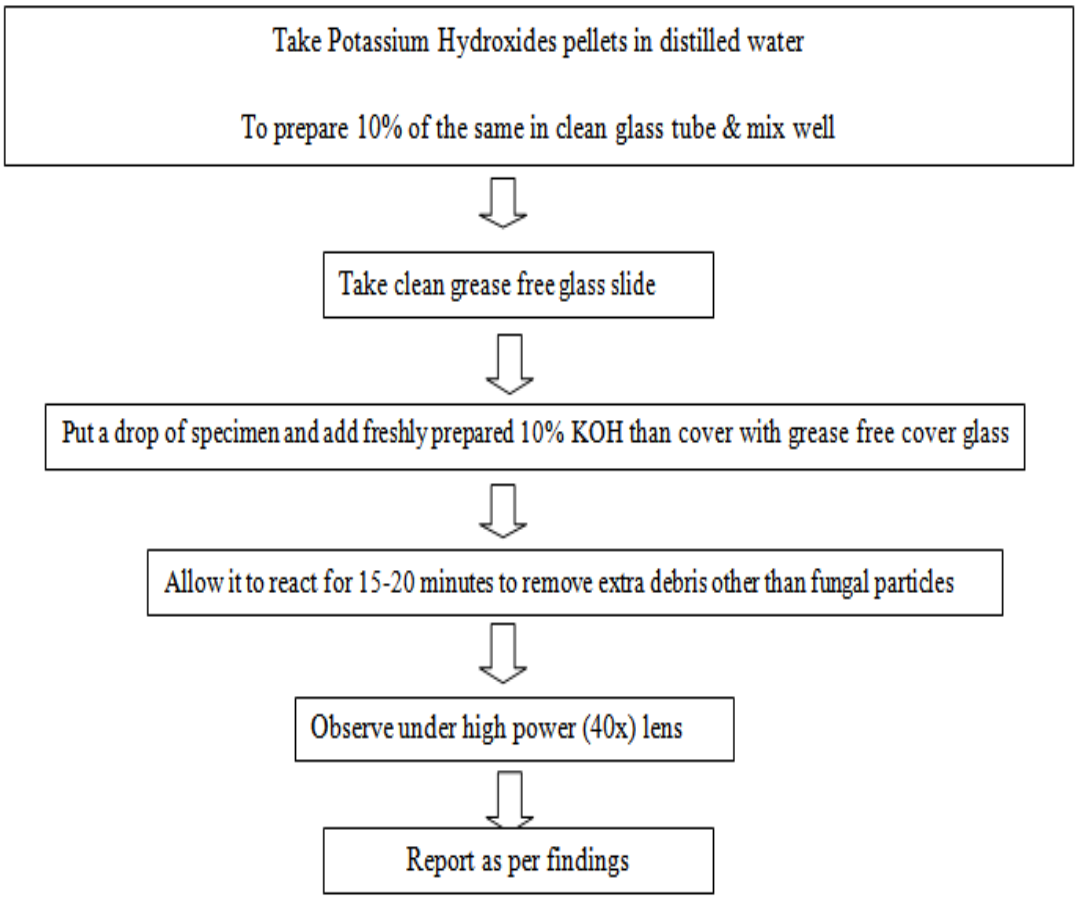
Procedure For 10% KOH Preparation



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 color 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 decolorization 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)[4]

Aim: To rule out any bacteriological findings.
Specimen: Abha Guggulu

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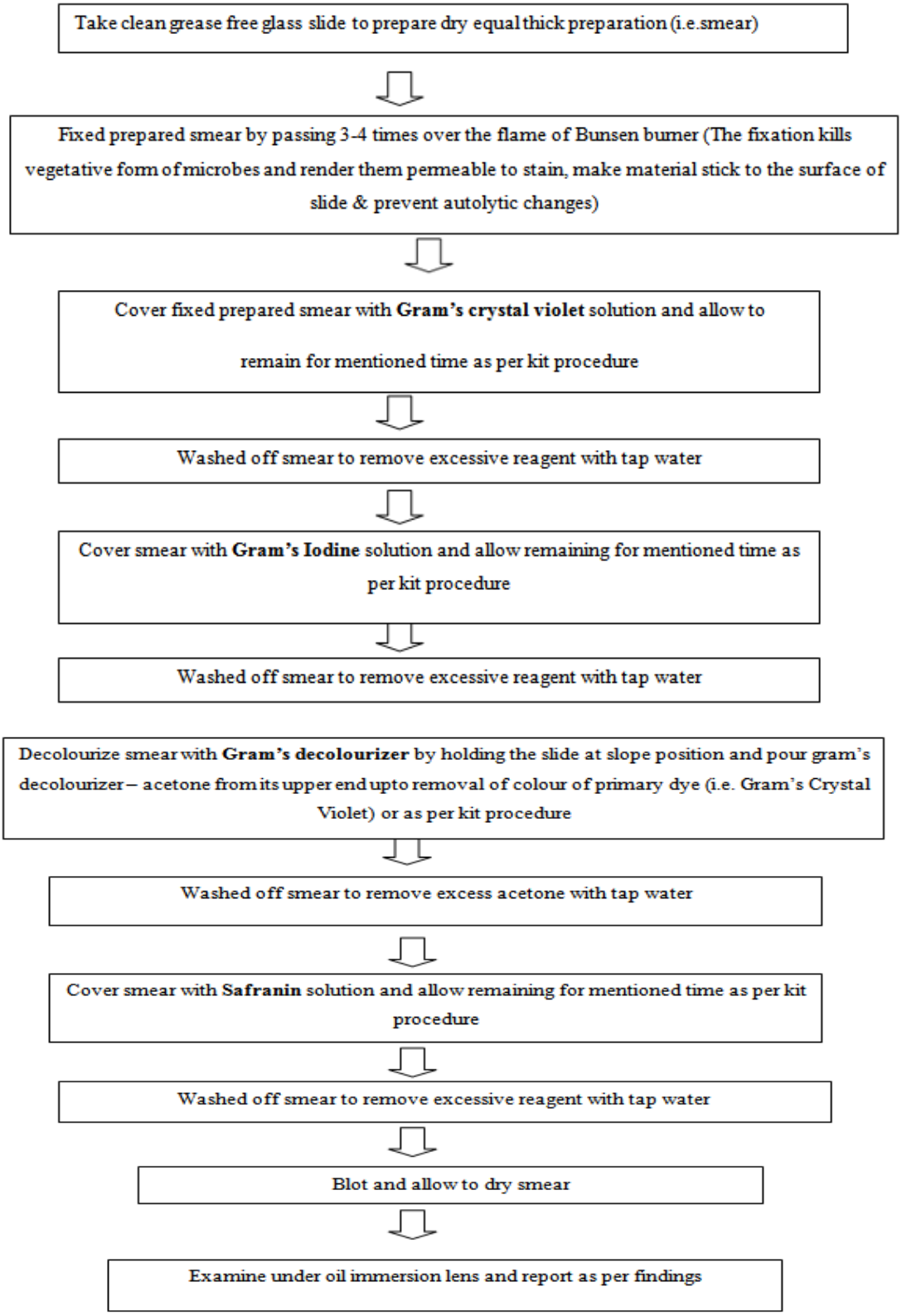
Procedure For Gram's Stain



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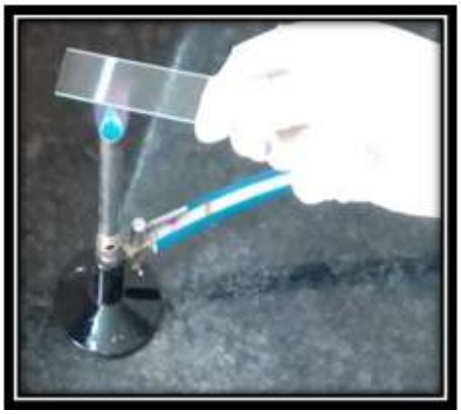


Figure 1.& 2. Smear staining Procedure

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.

Figure3. Sabouraud Dextrose Agar Base (SDA) bottle

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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 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. Oneloopful of the specimen is transferrd onto the onto the surface of well dried culture media]

↓

After selected incubation period examined growth by nacked 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.

↓

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

B. Aerobic Culture method

Company : HIMEDIA Laboratories Pvt. Ltd.

Required time duration : 24 to 48 hours

Required temperature : 37 °C

Use of media : for selective cultivation of pathogenic bacteria.

Figure 4.MacConkey Agar (MA)

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Procedure For Aerobic Culture

In the clinical microbiology laboratory culture method are employed for isolation of organism (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 specimen 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 cultured. One loopful of the specimen is transferred onto the surface of well dried plate]

↓

After streaking process **incubate** inoculated medium in inverted position at 37^0c for 18-24 hours in incubator under aerobic or 10% CO_2 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

III. OBSERVATIONS AND RESULTS

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

Results are shown in table no 2.

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Table 1: Showing observations of Abha Guggulu preserved at room temperature.

Sr. No.	Days of investigations After preparation of the sample	Temperature	Humidity	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	14 Days	36˚ C	69.4%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	46 Days	37˚ C	67.5%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	75 Days	30˚ C	65.1%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	106 Days	28˚ C	58.4%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	142 Days	31˚ C	58.6%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	177 Days	39˚ C	75.4%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	207 Days	38˚ C	62.3%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

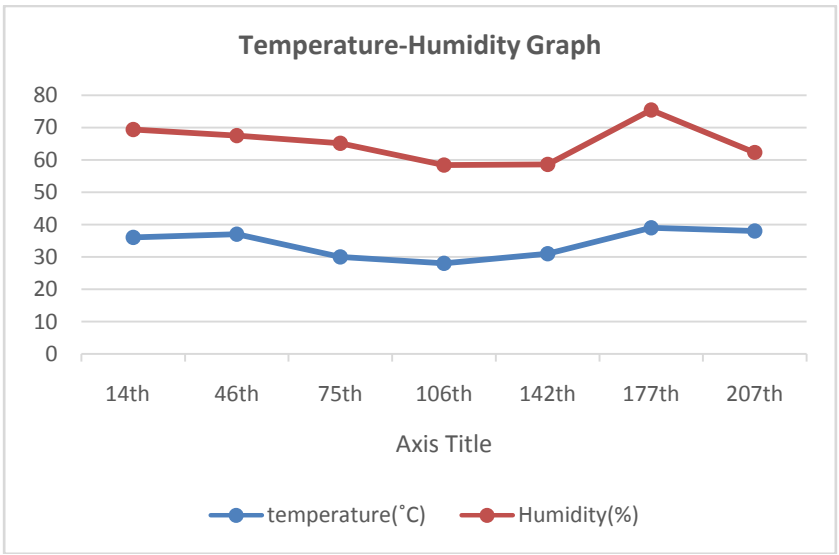

Figure 5. Temperature-Humidity Graph

IV. DISCUSSION:

Ayurveda as an adjuvant therapy is widely used in systemic disorders like Knee Osteoarthritis. Abha Guggulu is widely used in the treatment of Knee Osteoarthritis as it mainly acts on bones, joints and part of musculoskeletal system. It also

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has Agnideepana, Vatahara, shothahara and vedanastapana properties[5].In present study, it has shown a very good and promising result in reducing the symptoms of Knee Osteoarthritis. The present Study was carried out to observe the stability study of Abha Gugguluwith respect to Microbial Contamination of prepared sample and preserved in different climatic and temperature conditions. Thus, a baseline Microbial profile was studied up to the consumption of the prepared drug. At the end of study, it was found that sample was not contaminated with microbes.

Stability is usually expressed in term of shelf-life, which is the time period from when the product is produced until the time it is intended to be consumed or used. Microorganism needs water, humidity and temperature at suitable environmental conditions to develop in any media, surface or article.

V. 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 Abha Guggulu showed that the quality of Churna is in a standard condition. There were no growth found of microorganisms (bacterial or fungal),till 06th April 2018 i.e. 01 year& 03 months from the date of preparation, shows its good shelf life.

In the present study, Abha Guggulu, the final prepared drug shows stability shelf-life of approx.1½ year(Individual data as shown in table no. 2). Accordingly, maximum temperature found to be 39^{0}C and maximum relative humidity found to be 75.4% vice versa minimum temperature found to be 28^{0}C and minimum relative humidity found to be 58.4% during total study period.

Above mentioned data is a proven stability of prepared drug for Jamnagar region.

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