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STABILITY STUDY OF ASHWAGANDHADHI CHURNA IN TREATMENT OF KNEE OSTEOARTHRITIS - WITH RESPECT TO BASELINE MICROBIAL DIAGNOSTIC MODALITIES

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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. In present study, *Ashwagandhadi churna*, used for internal administration. In present study, stability with respect to its Microbial profile of all above mentioned drug is carried out. Drug was stored in plastic container during different climacteric conditions and were studied at regular intervals for a period of 6 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 microbes after 7 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.

KEYWORDS: *Ashwagandhadi churna*, Climate conditions, Microbial profile, Stability.

INTRODUCTION

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, Sandhigata vata* is one among *vata vyadhi* 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 by *Ashwagandhadi churna* 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.

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.

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.

MATERIALS AND METHODS

Sample of *Ashwagandhadi churna* 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 sample 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 I.T.R.A, Jamnagar. The ingredients and the part used are given in **(Table 1)**.

Table 1: Ingredients of *Ashwagandhadi curna(Anuboota yoga)*

Sl no	*Sanskrit* name/ English Name	Contents	Botanical name	Ratio
1	*Ashwagandha Churna*	*Ashwagandha*	Withania somnifera	1 part
2	*Pippalimoola churna*	*Pippalimoola*	Piper longum	1 part
3	*Rasayana Churna*	*Gokshura*	Tribulus terrestris	1/3 Part
		Guduchi	Tinospora cordifolia	1/3 Part
		Amalaki	Emblica officinalis	1/3 Part
4	*Chopachini Churna*	*Chopachini*	Smilax china	1 part
5	*Godanti bhasma*	*Godanti*	Dihydrate of Calcium sulphate	½ part

Date of Drug Preparation: 26th September, 2023.

Storage

Finished product of *Ashwagandhadi churna* 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.

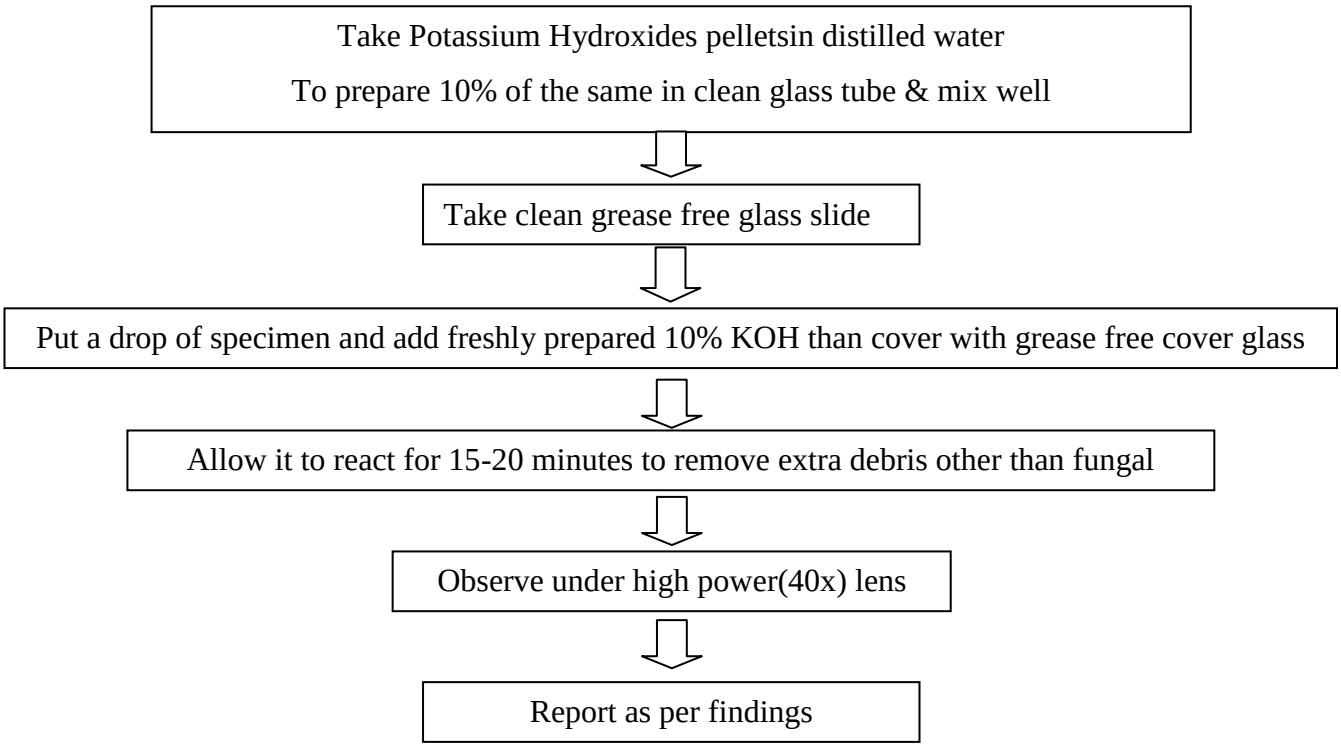
1. Smear Examination

A. 10% K.O.H. Preparation,

Aim: To rule out any mycological findings.

Specimen: *Ashwagandhadi churna*

Procedure For 10% KOH Preparation



B. Gram's stain test

Gram staining is a differential staining technique that differentiates bacteria into two groups: grampositive and gramnegative. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain procedure. Gramnegative bacteria are decolorized by any organic solvent (acetone or Gram's decolorizer) while Grampositive bacteria are not decolorized as primary dye retained by the cell and bacteria will remain as purple. After decolorizationstep, 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: *Ashwagandhadi churna*

Procedure For Gram's Stain

Take clean grease free glass slide to prepare dry equal thick preparation (i.e. smear)

⇩

Fixed prepared smear by passing 3-4 times over the flame of Bunsen burner (The fixation kills vegetative form of microbes and render them permeable to stain, make material stick to the surface of slide & prevent autolytic changes)

⇩

Cover fixed prepared smear with **Gram's crystal violet** solution and allow to remain for mentioned time as per kit procedure

⇩

Washed off smear to remove excessive reagent with tap water

⇩

Cover smear with **Gram's Iodine** solution and allow remaining for mentioned time as per kit procedure

⇩

Washed off smear to remove excessive reagent with tap water

⇩

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 excess acetone with tap water

⇩

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

⇩

Washed off smear to remove excessive reagent with tap water

⇩

Blot and allow to dry smear

⇩

Examine under oil immersion lens and report as per findings

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.

Figure 3: 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 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

Respected materials collected with sterile cotton swab for inoculation purpose on selected aerobic 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.

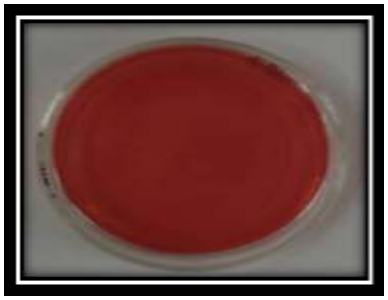


Figure 4: MacConkey Agar (MA).

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 sreaks 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 nacked eye in form of colony and **confirm growth** by performing different related biochemical reactions and different related staining procedures.

After that **report** isolates

OBSERVATIONS AND RESULTS

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.

Results are shown in table no 2.

Table 2: Showing observations of *Ashwagandhadi churna* 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%	Microorganismxs 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

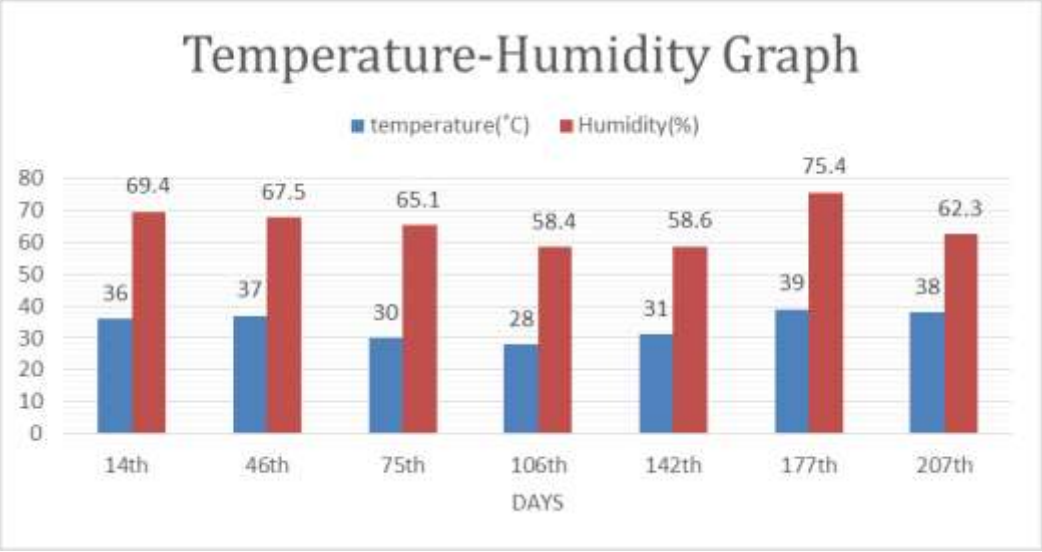


Figure 4: Temperature and Humidity Graph.

DISCUSSION

Ayurveda as an adjuvant therapy is widely used in Musculoskeletal disorders like Knee Osteoarthrirts. *Ashwagandhadi churna*- the combination of *Ashwagandha churna, Rasayana Churna, Pippalimoolachurna, Chopachini churna* in equal quantity was never used before for the research work at ITRA and elsewhere in India in Knee Osteoarthritis. Antioxidant and

anti inflammatory effect of *Ashwagandha churna, Vatahara, Deepana* and *Pachana e*ffect of *pippalimoola churna, Shoolagna* effect of *Chopachini churna* and mixture *of Guduchi, Amalaki, Gokshura churna* has the *Rasayana* effect[5] together contributes in alleviating the symptoms of Knee Osteoarthritis. 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 *Ashwagandhadi churna*, with respect to Microbial Contamination of prepared sample and preserved in different climatic and temperature conditions. Thus a baseline Microbial profile was studied at regular interval of 1 month for 6 months. At the end of study it was found that sample was not showed presence of any 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.

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 *Ashwagandhadi churna* showed that the quality of *Churna is* in a standard condition. There were no growth found of microorganisms (bacterial or fungal), till 18th April 2024 i.e. 7 months from the date of preparation, shows its good shelf life.

In the present study, *Ashwagandhadi churna*, 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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