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Original Research Article

STABILITY STUDY OF *RASNAPANCHAKA KWATHA*,USED IN TREATMENT OF POST-CHIKUNGUNYA CHRONIC INFLAMMATORY RHEUMATISM - WITH RESPECT TO BASELINE MICROBIAL DIAGNOSTIC MODALITIES

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ABSTRACT

Chikungunya fever (CHIK) is an emerging, mosquito-borne disease caused by an alphavirus, Chikungunya virus(CHIKV) that cause s fever and debilitating joint pains in humans. It is vectored primarily by the tropical and sub-tropical mosquito but Aedes aegypti is also found to be transmitted by Aedes albopictus. Studies shows that musculoskeletal problem remain present for weeks, months and years after onset of chikungunya fever. *Rasnapanchaka kwatha* mentioned in *Bhaisajyaratnavali* as a therapeutic formulation to treat *Asthi-Majjagata Vata*. *Rasnapanchaka kwatha* was used to as *shamana* therapy in Post-Chikungunya Chronic Inflammatory Rheumatism. **Aims:** To carried out stability of coarse powder of *Rasnapanchaka kwatha* with respect to its microbial profile. **Materials and Methods:** Sample of Coarse powder of *Rasnapanchaka kwatha* was prepared and studied to check microbial contamination at regular intervals. **Results:** Every time sample was subjected to the microbiological study from the date of the preparation to the date of last microbiological study. No any contaminations was found in microbiological study. **Discussion:** Hence the present Study was carried out to observe the stability study of coarse powder of *Rasnapanchaka kwatha* with respect to Microbial Contamination of sample prepared and preserved in different climatic and

temperature conditions. Thus a baseline Microbial profile was studied at regular interval as consumption of drug for patient's usage. At the end of study it was found that sample was not showed presence of any Microbes. **Conclusion:** At the end of study *Rasnapanchaka kwatha* container has not present of microbes after 15 month of preparation sample, even in different climate and temperature. Hence in present study the stability test of *Rasnapanchaka kwatha* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEY WORDS: Stability, Microbial profile, Chikungunya, Post-Chikungunya Chronic Inflammatory Rheumatism**,** *Rasnapanchaka kwatha*, Climate conditions.

INTRODUCTION:

Chikungunya virus (CHIKV) is an alphavirus from the *Togaviridae* family that causes acute arthropathy in humans and other animals.[i] [ii] Transmission usually starts with an infected mosquito bite after which the virus infects fibroblasts and macrophages in the dermis.[iii] After an incubation period of 3–7 days, it is disseminated through the lymphatic system and blood- stream to epithelial and endothelial cells, and other tissues and cells.[iv] The virus replicates causing viraemia, fever, rash, myalgia, arthralgia, and arthritis.[v] At this point, the acute phase is established, lasting for approximately 2 weeks and characterized by the appearance of immunoglobulin type M (IgM) (persisting for up to 3 months) followed by the production of immunoglobulin type G (IgG), which provides anti- viral immunity for years.[vi] After the acute phase, CHIKV infection can progress to a chronic stage where rheumatic symptoms can last for several months to years. [vii] In May 2015, the World Health Organization defined a person with chronic chikungunya as a "person with previous clinical diagnosis of chikungunya after 12 weeks of the onset of the symptoms presenting with at least 1 of the following articular manifestations: pain, rigidity, or edema, continuously or recurrently."[viii] Chronic arthralgia (i.e., "suspected chronic chikungunya arthritis" or "CCA") is a common complication post CHIKV-infection. Some studies[ix] have reported that 40 to 60% of patients with acute chikungunya experience significantly impaired quality of life in the long term.

In later stage of Post chikungunya there is seen *Vata* and *Kapha* Dosha dominancy. A compound formulation is prepared by selecting the drugs having specific action against the *Doshas* involved in the pathogenesis of disease. *Rasnapanchaka kwatha* is an important compound formulation mentioned in Ayurvedic classics for diseases of various *sandhi- Asthi-Majja gata vatam*, also in conditions of *Sarvangavatam vatam*. *Rasnapanchaka kwatha* mentioned in *Bhaisajyaratnavali* in *Amvat chikitsa*[x]. Contain of *Rasnapanchaka kwatha* having *Katu*, *Tikta Rasa* therefor it is usful for *Dosha Pachan* who is elevated. For the first time the research work carried out for its authentication and microbial profile. The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar by adopting standard operative procedure for *kwatha* formation. No any preservative was added to the test drug. Drug preparation was finished on 13/10/2020.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 ,chamical, microbiological therapeutic specifications. Thus in the present study on attempt was taken to check stability of *kwatha* with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval.

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 *Rasnapanchaka kwatha* was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals. 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 346th day of preperation, Before giving *kwatha* to the patients. Then samples of *Rasnapanchaka kwatha* was subjected to the microbilogical 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 *Rasnapanchaka kwatha*[xi]

Sr.no.	Contents	Latin name	Part used	Part
1.	*Rasna*	*Pluchea lanceolata DC.*	Dried Root	1
2.	*Guduchi*	*Tinospora cordifolia Willd*	Dried Stem	1
3.	*Erand*	*Ricinus communis Linn.*	Dried root	1
4.	*Devadaru*	*Cedrus deodara* Roxb.	Stem	1
5.	*Shunthi*	*Zingiber officinale* Roxb.	Rhizome	1

Date of Drug Preparation: 13th October 2020

Storage:

Finished product of *Rasnapanchaka kwatha* 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) Wet mount /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.

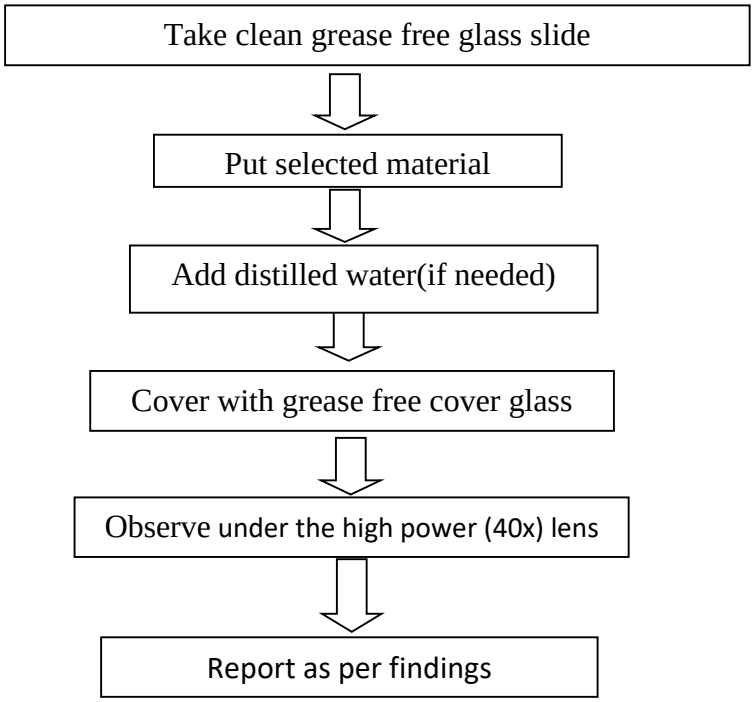
1. Smear Examination:

A. Wet mount /10% K.O.H. Preparation:

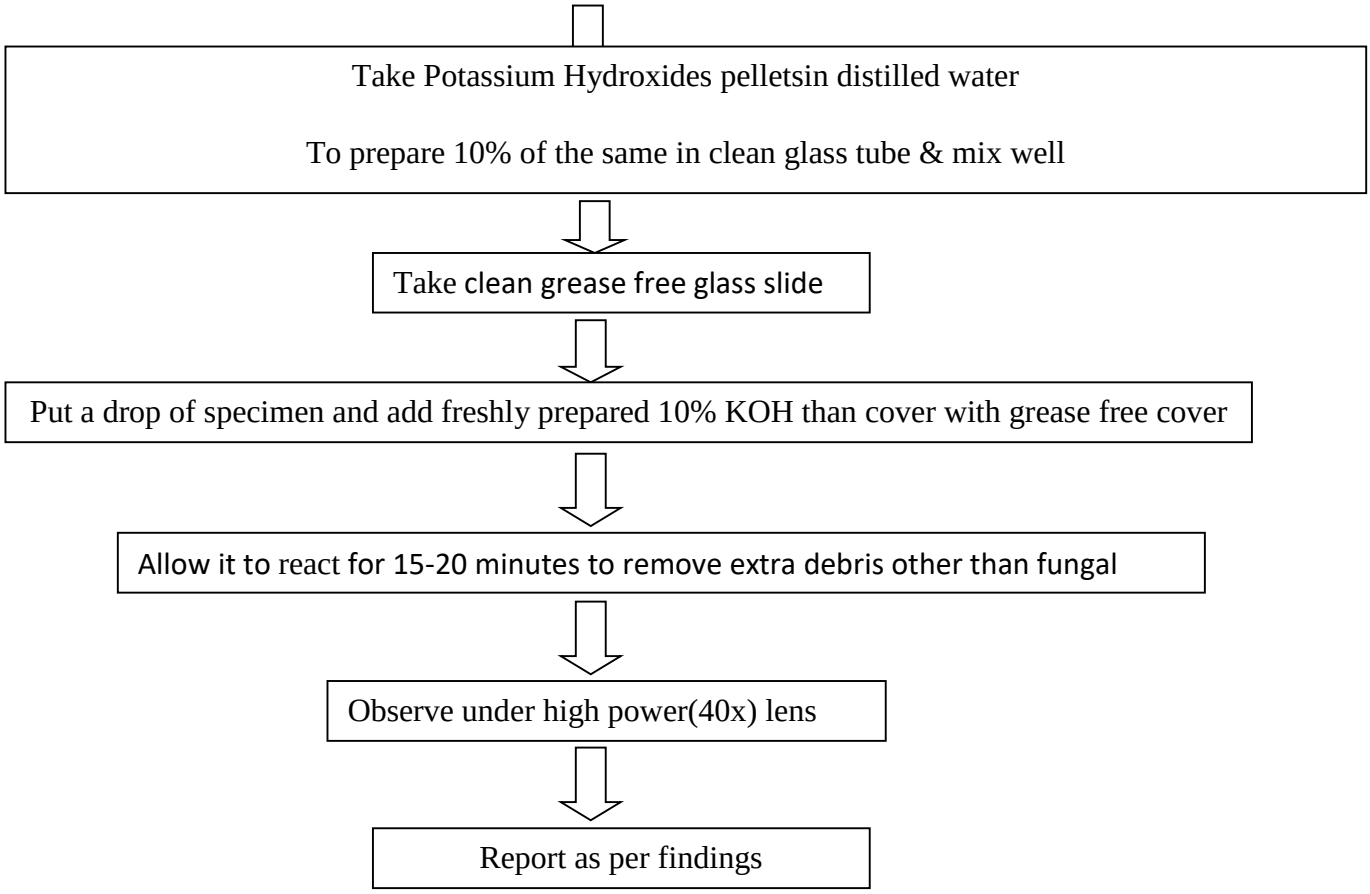
Aim: To rule out any mycological findings.

Specimen:*Rasnapanchaka kwath*

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 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)[xii]

Aim: To rule out any bacteriological findings.

Specimen: ***Rasnapanchaka kwath***

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

⇩

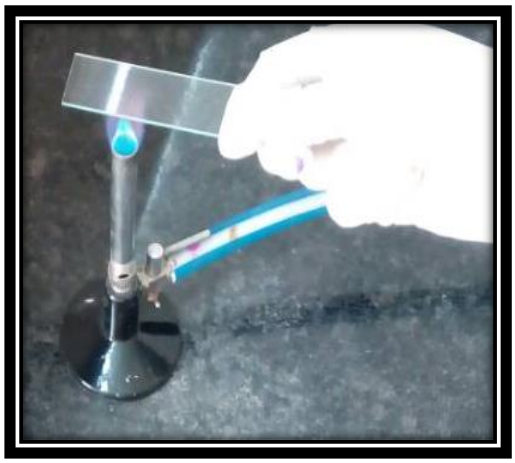
Cover smear with **Safranin** solution and allow remaining for mentioned time as per kit procedure

⇩

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



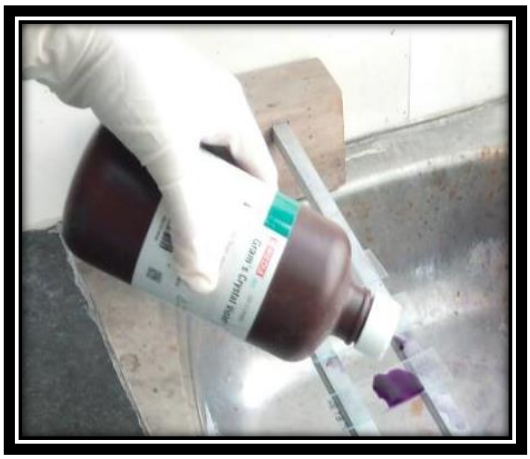


Figure 1.& 2. Smear staining Procedure

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

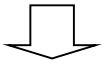
Use of media : For selective cultivation of pathogenic fungi.

Figure3. Sabouraud Dextrose Agar Base (SDA) bottle

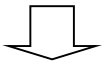
Procedure For Fungal Culture

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)

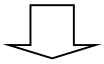


Choose appropriate selective solid media for inoculation purpose

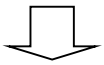


Dry selective solid media in Hot Air Oven **before** specimen inoculation

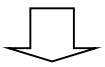
Allow to cool dried medium before **Specimen inoculation**



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 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 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.

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.

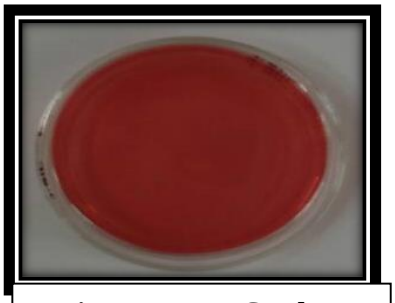


Figure 4.MacConkey Agar (MA)

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

Results are shown in table no 2.

Table 2: Showing observations of sample preserved at room temperature.

Sr. No.	Days of investigations After preparation of the sample	Temprature	Humidity	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	346 Days	25˚ C	95%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	361 Days	27˚ C	60%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	376 Days	30˚ C	75%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	391 Days	33˚ C	20%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	406 Days	30˚ C	25%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	421 Days	26˚ C	40%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

7.	**451 Days**	**19˚ C**	**47%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

Discussion: Ayurveda as an adjuvant therapy is widely used in musculo-skeleton disorders like Chikungunya and Post-Chikungunya Chronic Inflammatory Rheumatism. *Rasnapanchaka kwatha* is never used before for the research work for clinically diagnosed/confirmed patient of Post-Chikungunya Chronic Inflammatory Rheumatism at panchakarma O.P.D., I.T.R.A. and elsewhere in India. *Rasnapanchaka kwath* used for *Shamana therapy* in Post-Chikungunya Chronic Inflammatory Rheumatism was processed with *Tikta-Katu Rasa Pradhan Dravya* which has *Aampachana* properties, which is useful in this condition in present study. In present study it has shown favourable result in Post-Chikungunya Chronic Inflammatory Rheumatism. Hence the present Study was carried out to observe the stability study of *Rasnapanchaka kwatha* with respect to Microbial Contamination of sample prepared and preserved in different climatic and temperature conditions. Thus a baseline Microbial profile was studied at regular interval as consumption of drug for patient's usage. At the end of study it was found that sample was not showed presence of any Microbes (either bacterial or fungal).

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 the *Rasnapanchaka kwatha* showed that the quality of *kwatha* is in a standard condition. There were no growth found of microorganisms (bacterial or fungal),till 12th January 2022 i.e. 01 year & 03 months from the date of preparation, shows its good shelf life.

Stability of tested drug proven at maximum 33˚ C temperature with 20% humidity and at minimum 19˚ C temperature with 47% humidity and all over maximum humidity responsible for drug stability is 95% and minimum humidity responsible for drug stability is 20% and all over maximum temperature responsible for drug stability is 33˚ C and minimum temperature responsible for drug stability is 19˚ C.

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