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# "EVALUATION OF MICROBIAL STABILITY OF *DESMODIUM TRIQUETRUM* (L.) DC. USED IN THE MANAGEMENT OF *KASA* UNDER VARYING TEMPERATURE AND RELATIVE HUMIDITY CONDITIONS"

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**Abstract:**

**Background:** *Desmodium triquetrum* (L.) DC. is a therapeutically valuable drug used in the management of *Kasa* (cough), attributing to its diverse phytoconstituents and pharmacological activities. Although *Kwatha* is the intended therapeutic dosage form, it is traditionally prepared freshly prior to each administration. Hence, the drug is stored in the form of *Yavakuta* (coarse powder), making its microbiological stability during storage an important consideration for maintaining the safety and quality of the decoction prepared for therapeutic use. **Aim:** To assess the microbiological stability of *Desmodium triquetrum Panchanga Yavakuta* during storage and determine the extent of microbial contamination in freshly prepared *Kwatha* at predetermined time intervals under varying temperature and relative humidity conditions. **Materials and Methods**: Ten kilograms of *Desmodium triquetrum Panchanga* was collected from the Nilambur forest region of Kerala, shade-dried, processed into *Yavakuta*, and stored in sterile, airtight containers at room temperature. The *Kwatha* was freshly prepared following the classical method by adding 16 parts of water and reducing the volume to one-eighth. Microbiological evaluation was carried out in the ITRA Microbiology Laboratory using Gram staining, 10% KOH wet mount examination, aerobic bacterial culture, and fungal culture. Samples were evaluated at monthly intervals over a period of 12 months under ambient temperature conditions ranging from 22.2–38°C and relative humidity ranging from 34–80%. **Results:** Initial microbiological examination of the stored *Yavakuta* revealed the presence of *Aspergillus flavus* and *Penicillium* species, which were considered commensal organisms. The *Yavakuta* was subsequently

subjected to dry-heat sterilization, following which freshly prepared *Kwatha* samples were evaluated. No bacterial or fungal growth was detected in the *Kwatha* samples throughout the 12-month observation period, despite seasonal variations in temperature and relative humidity. Repeated monthly microbiological assessments consistently demonstrated satisfactory microbiological quality. **Conclusion:** *Desmodium triquetrum Panchanga Yavakuta* demonstrated satisfactory microbiological stability under the studied storage conditions. Following dry-heat sterilization and preparation as fresh *Kwatha*, no detectable bacterial or fungal contamination was observed throughout the study period. These findings support the traditional practice of storing the drug in *Yavakuta* form and preparing *Kwatha* freshly before administration, thereby contributing to the maintenance of its microbiological quality and safety.

**Keywords:** *Desmodium triquetrum*, *Yavakuta*, *Kwatha*, microbial stability, *Saviryata Avadhi*, quality control

## INTRODUCTION

*Desmodium triquetrum* (L.) DC. [syn. *Tadehagi triquetrum* (L.) H. Ohashi] is a medicinal plant belonging to the family Fabaceae and is widely distributed in tropical and subtropical regions of Asia. It has a long history of use in traditional medicine, particularly in several regions of China, Myanmar and other parts of Southeast Asia. Different parts of the plant have traditionally been used for the management of respiratory, gastrointestinal, hepatic, inflammatory and other disorders.[1,2]

The medicinal importance of *D. triquetrum* is attributed to its diverse phytochemical composition. Phytochemical investigations have reported the presence of flavonoids, prenylated isoflavonoids, phenylpropanoids, phenolic compounds, triterpenoids, steroids and other secondary metabolites. Compounds such as triquetrumones, kaempferol, astragalin, ursolic acid, betulinic acid, beta-sitosterol, stigmasterol and daucosterol have been identified from different parts of the plant.[3,4] The occurrence of these structurally diverse constituents provides a possible basis for the broad pharmacological activities reported for the plant.

Several experimental studies have demonstrated the pharmacological potential of *Desmodium triquetrum*. Extracts and isolated constituents have exhibited anti-inflammatory, antimicrobial, hepatoprotective, antihyperlipidemic, antioxidant, anticancer, hypoglycemic, and cerebroprotective activities.[5,6,7,8,9,10]

The traditional use of *D. triquetrum* is particularly relevant to respiratory disorders. Ethnomedicinal literature reports the use of the plant in respiratory ailments, including cough. In Myanmar, a decoction prepared from the roots has traditionally been used for chronic cough and tuberculosis.[11] Recent literature has additionally documented its traditional use in respiratory infections and reported pharmacological activities relevant to respiratory disorders, including anti-tussive, anti-histaminic, mast cell degranulation, anti-inflammatory and antimicrobial effects.[12,13] These observations provide a rationale for further investigation of *Desmodium triquetrum* in relation to respiratory conditions such as *Kasa* (cough).

The *Panchanga* (whole plant) of medicinal plants is used in various Ayurvedic preparations, including *Kwatha*. *Kwatha* is an important dosage form described under *Panchavidha Kashaya Kalpana*, in which the coarse powder of the drug is boiled with water to obtain the aqueous decoction.[14] In the present study, *D.*

*triquetrum Panchanga* is maintained in the form of *Yavakuta* (coarse powder), which serves as the pharmaceutical raw material for preparation of the decoction.

Traditionally, *Kwatha* is intended for fresh preparation and administration and is generally not intended for prolonged storage. Therefore, the *Panchanga* stored as *Yavakuta* assumes importance as the raw material from which the decoction is freshly prepared before administration according to the prescribed method.[15] Although dried herbal material contains substantially less available water than an aqueous preparation, it may still undergo microbial contamination during collection, drying, pulverization, handling, packaging and storage.[16] Consequently, maintenance of the microbiological quality of stored *D. triquetrum Panchanga Yavakuta* is important for ensuring the quality and safety of the freshly prepared *Kwatha*.

The concept of stability may be considered in relation to the classical Ayurvedic concept of *Saviryata Avadhi*, referring to the period during which a medicinal preparation is expected to retain its intended quality and therapeutic potency. Various Ayurvedic texts have described specific periods of use for different dosage forms, while scientific studies have attempted to evaluate the shelf life of Ayurvedic formulations using appropriate quality parameters.[17] Thus, scientific evaluation of stability parameters can provide objective evidence regarding the quality and safety of medicinal preparations during storage.

In modern pharmaceutical science, stability refers to the ability of a pharmaceutical product to retain its specified physical, chemical, microbiological, therapeutic and toxicological characteristics throughout its intended storage period.[18] Microbial survival and multiplication in herbal materials are influenced by several environmental and intrinsic factors, including temperature, relative humidity, moisture availability, pH, nutrient availability, storage duration and handling conditions.[19]

Microbiological analysis provides an objective means of evaluating the microbial quality and stability of herbal materials. It may include assessment of total viable aerobic microbial count and fungal count, together with detection of specified or objectionable microorganisms. Such examination helps to determine whether microbial contamination increases, decreases or remains within acceptable limits during storage.[20] The World Health Organization recognizes microbiological quality assessment as an important component of quality control of medicinal plant materials and herbal products.[21]

Therefore, assessment of the microbial stability of *D. triquetrum Panchanga Yavakuta* under varying temperature and relative humidity conditions is relevant for establishing its microbiological quality and storage stability. Such evaluation may also provide useful information regarding the quality of the raw material used for preparation of *Kwatha* and the influence of environmental conditions on its microbial stability.

**AIM**

To assess the microbiological stability of *Desmodium triquetrum Panchanga Yavakuta* during storage and determine the extent of microbial contamination in freshly prepared *Kwatha* at predetermined time intervals under varying temperature and relative humidity conditions.

**OBJECTIVE**

To ensure adherence to standardization and quality control measures in the preparation and storage of *Desmodium triquetrum Yavakuta* by evaluating its microbial profile throughout the study period.

## MATERIALS AND METHODS

**Details of Drug Collection: -**

**Botanical name of Drug:** *Desmodium triquetrum* (L.) DC.

**Month of collection:** May 2025 (Collection completed on 23rd May 2025)

**Quantity**:10 kg

**Geographical details of the habitat:** Nilambur Forest, Kerala

**Latitude:** 11º 15' 46.08'' N

**Longitude:** 76º 18' 49.32'' E

**Processing of Drug: -**

The collected whole plant material of *Desmodium triquetrum* (L.) DC. was examined and separated from extraneous matter, followed by thorough cleaning and shade drying. The dried material was coarsely powdered using sieve no. 40 to obtain *Yavakuta*. The prepared *Yavakuta* was packed in sterile containers and stored at room temperature.

As the intended dosage form was *Kwatha* (decoction), which is classified as a *Sadya Prayoga Kalpana*, the decoction was prepared freshly before each administration. Accordingly, the material used for microbiological evaluation was the freshly prepared *Kwatha*. The decoction was prepared according to the classical method by adding 16 parts of water to the coarse powder and heating till bubbling with temperature reaching to approximately 121°C, until the volume was reduced to one-eighth of the initial quantity (approximately 20 minutes), followed by straining. The prepared decoction was subsequently subjected to microbiological examination at regular intervals over a period of 12 months.

The microbiological evaluation was carried out in the Microbiology Laboratory, Institute of Teaching and Research in Ayurveda (I.T.R.A.), Jamnagar. Two sets of microbiological assessments were performed to evaluate the presence of bacterial and fungal contamination in the drug, particularly in its final dosage form, i.e., *Kwatha*.

The initial microbiological assessment was performed on the 46th day after completion of collection, following adequate drying and preparation of the *Yavakuta*. At this stage, the *Yavakuta* was examined microbiologically and was subsequently subjected to dry-heat sterilization. Thereafter, the final dosage form, i.e., *Kwatha*, was freshly prepared from the sterilized material from the same storage container and subjected to microbiological examination at monthly intervals across different seasons. Microbiological stability was monitored from July 2025 to June 2026 under ambient storage conditions. During the study period, the recorded temperature ranged from 22.2–38°C, while relative humidity ranged from 34–80%.

**Storage Conditions: -**

The *Yavakuta* of *Desmodium triquetrum Panchanga* was stored in an airtight, food-grade plastic container at room temperature in a well-lit area, without exposure to direct sunlight. A clean and dry stainless-steel spoon was used for dispensing the drug to minimize the risk of external contamination.

**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) <u>PROCEDURE FOR 10% KOH PREPARATION:</u>**

i. Take Potassium Hydroxides pellets (of HiMedia Lab. Pvt. Ltd.) in distilled water to prepare 10% of the same in clean glass tube & mix well
ii. Take clean grease free glass slide
iii. Put a-drop of specimen and add freshly prepared 10% KOH then cover with grease free cover glass
iv. Allow it to react for 15-20 minutes to remove extra debris other than fungal particles
v. Observe under high power (40x) lens
vi. Report as per findings

**B) <u>PROCEDURE FOR GRAM'S STAIN TEST:</u>**

i. Take clean grease free glass slide to prepare dry equal thick preparation (i.e. smear)
ii. 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)
iii. Cover fixed prepared smear with Gram's crystal violet solution and allow to remain for mentioned time as per kit procedure
iv. Washed off smear to remove excessive reagent with tap water
v. Cover smear with Gram's Iodine solution and allow to remain for mentioned time as per kit procedure
vi. Washed off smear to remove excessive reagent with tap water
vii. Decolorize smear with Gram's decolorizer by holding the slide at slope position and pour gram's decolorizer acetone from its upper end upto removal of colour of primary dye (i.e. Gram's Crystal violet) or as per kit procedure
viii. Washed off smear to remove excess acetone with tap water
ix. Cover smear with Safranin solution and allow to remain for mentioned time as per kit procedure
x. Washed off smear to remove excessive reagent with tap water

xi. Blot and allow to dry smear

xii. Examine under oil immersion lens and report as per findings

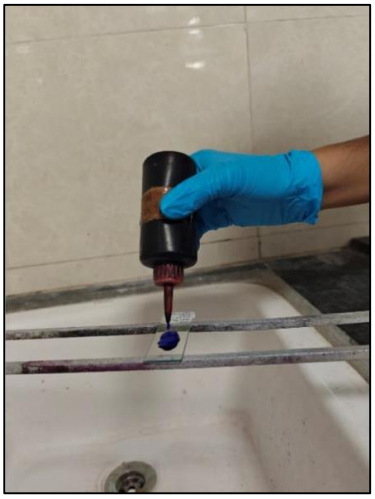

Figure 1 – Smear Staining Procedure

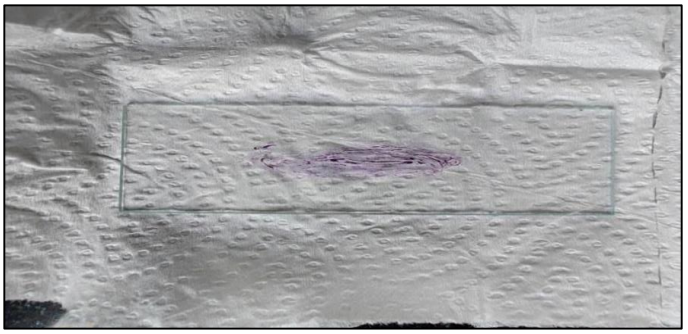

Figure 2 – Stained Smear

2**. CULTURE STUDY: -**

**A) FUNGAL CULTURE METHOD:**

Respective 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 °C

Use of media: For selective cultivation of pathogenic fungi.

**Procedure For Fungal Culture:**

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

ii. Choose appropriate selective solid media for inoculation purpose

iii. Inoculate selected specimen 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]

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

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

vi. 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

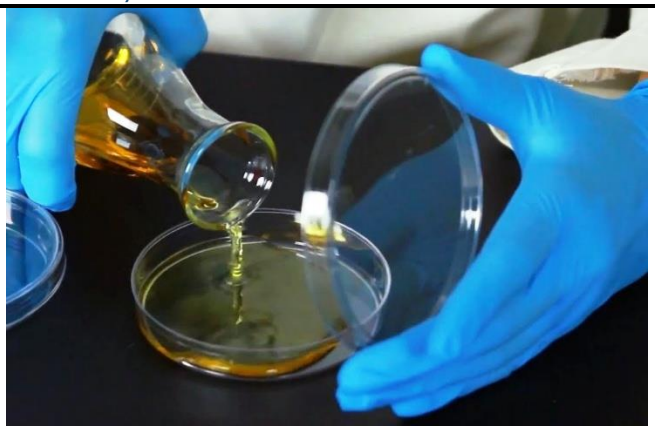

Figure 3 – Fungal culture media preparation with Sabouraud Dextrose Agar Base (SDA)

**B) AEROBIC CULTURE METHOD**

Respective materials were 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 °C

Use of media: For selective cultivation of pathogenic bacteria

**Procedure For Aerobic Culture: -**

i. In the clinical microbiology laboratory, culture methods are routinely employed for the isolation of microorganisms. The streak culture method was used for obtaining isolated colonies.

ii. Appropriate selective solid culture media were selected according to the requirements of the specimen and the microorganisms under investigation.

iii. The selected solid media were dried in a hot-air oven before inoculation. The media were subsequently allowed to cool to room temperature before inoculation.

iv. The selected specimen was inoculated onto the surface of the cool, dried culture medium by the four-flame streaking method using a sterile Nichrome wire loop (24 S.W.G.). The loop was first sterilized in the oxidizing zone of the Bunsen burner flame (blue flame) and allowed to cool. It was then charged with the selected specimen, and one loopful of the specimen was transferred onto the surface of the culture medium. The specimen was streaked systematically over the agar surface to obtain isolated colonies. The loop was flamed and allowed to cool between successive sets of streaks, resulting in four flame cycles.

v. After inoculation, the culture plates were incubated in an inverted position at 37°C for 18–24 hours in an incubator under appropriate atmospheric conditions, i.e., aerobic or 10% CO2 atmosphere, as required for the selected culture conditions.

vi. Following the prescribed incubation period, the inoculated media were examined for visible microbial growth in the form of colonies by naked-eye observation. The isolated colonies were further subjected to appropriate staining procedures and relevant biochemical tests for identification and confirmation of the isolates.

vii. Based on the colony morphology, staining characteristics, and biochemical reactions, the isolated microorganisms were identified and recorded accordingly.

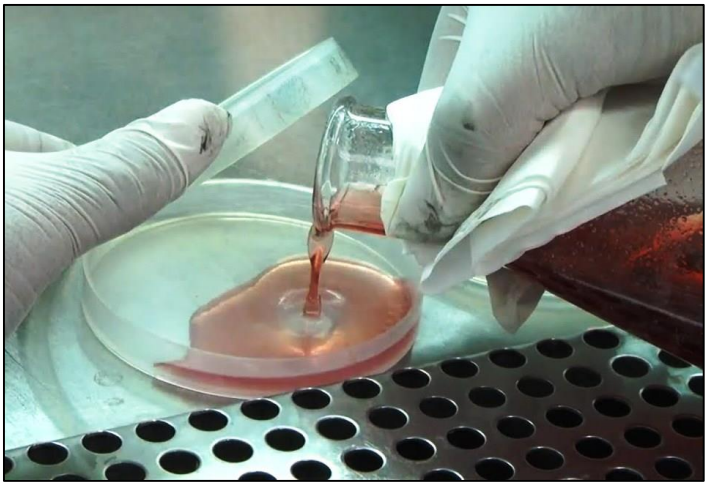

Figure 4 – Aerobic culture media preparation with MacConkey Agar (MA)

## RESULTS

The samples were subjected to microbiological evaluation at monthly intervals from the date of preparation until the completion of the final microbiological assessment. The results obtained are presented in Table 1.

**Table 1: Showing microscopic profile of samples preserved at room temperature**

**(Drug Collection Completion Date: 23/05/2025)**

| Sr. No. | Date and duration after collection of raw material (days) | Temperature (˚C) | Relative Humidity (RH) (%) | Microbiological Observations | | | |
|---|---|---|---|---|---|---|---|
| | | | | Gram's Stain | Aerobic culture | Wet mount/ 10% KOH | Fungal culture |
| *Desmodium triquetrum* (L.) DC. *Panchanga Yavakuta* | | | | | | | |
| 1. | 07/07/2025 46th Day | 29.4˚C | 78% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | Aspergillus flavus & Penicilliu m species isolated |
| *Desmodium triquetrum* (L.) DC. *Panchanga Kwatha* | | | | | | | |
| 2. | 10/07/2025 49th Day | 29.4˚C | 78% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 3. | 11/08/2025 81st Day | 28.3˚C | 80% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 4. | 15/09/2025 116th Day | 28.3˚C | 77% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 5. | 16/10/2025 147th Day | 29.4˚C | 56% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 6. | 10/11/2025 172nd Day | 26.1˚C | 47% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 7. | 18/12/2025 210th Day | 22.2˚C | 48% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 8. | 19/01/2026 242nd Day | 28˚C | 52% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 9. | 17/02/2026 271st Day | 30˚C | 47% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 10. | 16/03/2026 298th Day | 33˚C | 34% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 11. | 15/04/2026 328th Day | 34˚C | 40% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 12. | 19/05/2026 362nd Day | 38˚C | 50% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| 13. | 15/06/2026 389th Day | 33˚C | 69% | Micro-organisms not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |

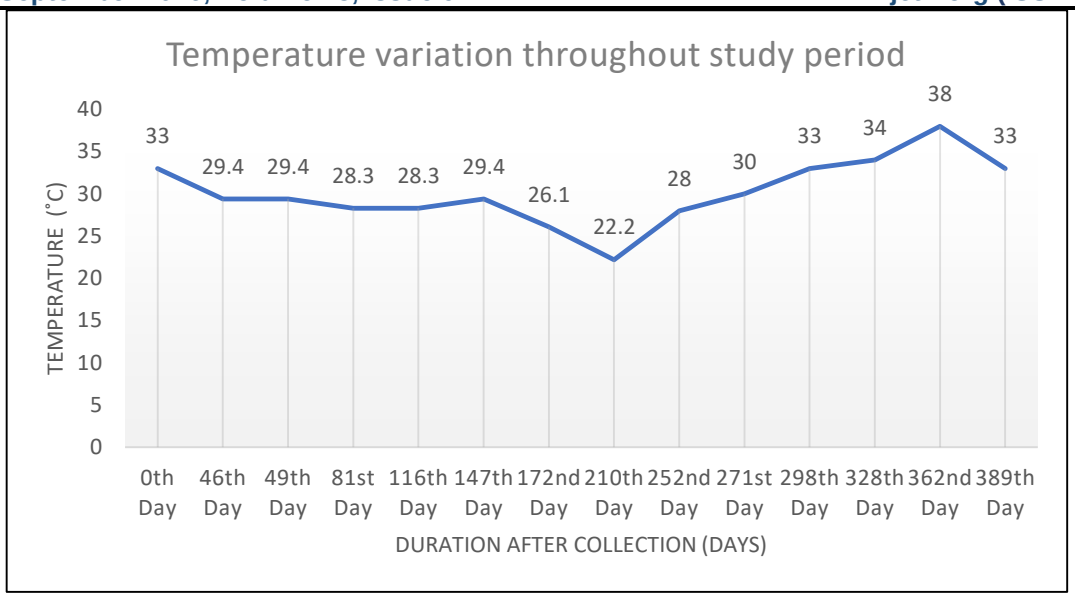


Figure 5 – Variation in Temperature Throughout the Study Period

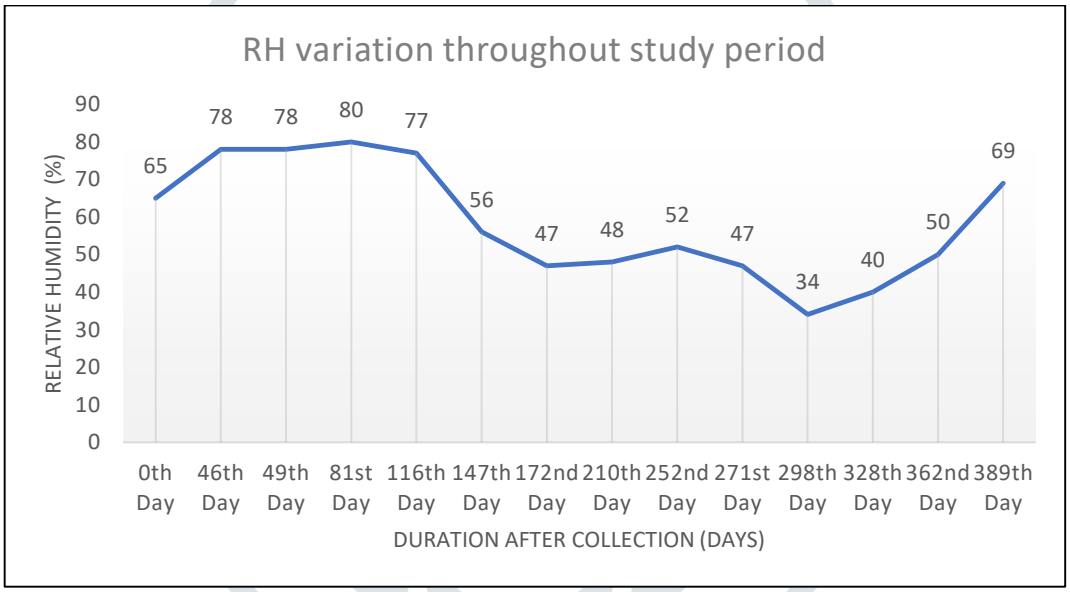


Figure 6 – Variation in Relative Humidity Throughout the Study Period

## DISCUSSION

The therapeutic efficacy of *Desmodium triquetrum* (L.) DC. in the management of *Kasa* (cough) has been demonstrated through research conducted at ITRA, Jamnagar. Considering that the quality and safety of herbal medicines are closely associated with their microbiological status, the present study was undertaken to assess the microbial stability of *Desmodium triquetrum* under routine storage conditions.

The microbiological quality of herbal medicines is of considerable importance, as raw medicinal plant materials may acquire environmental microorganisms at various stages, including cultivation, harvesting, transportation, processing, and storage. The proliferation of microorganisms in herbal drugs is influenced by several factors, such as moisture content, temperature, relative humidity, handling practices, storage conditions, and the inherent microbial flora associated with the plant material.[22] Environmental conditions characterized by increased humidity, particularly when combined with inadequate storage conditions, may further favour microbial contamination and proliferation in herbal preparations.[23]

The plant material utilized in the present study was collected from the Nilambur forest of Kerala and subsequently stored at Jamnagar, Gujarat, a coastal region where climatic conditions are characterized by comparatively high humidity, particularly during the monsoon season. [24] Such environmental conditions were considered relevant while evaluating the microbial stability of the stored plant material. Temperature and relative humidity are recognized as important factors affecting microbial growth; therefore, the variations observed during the study period, with temperature ranging from 25–41 °C and relative humidity from 34–80%, were taken into consideration during the assessment of microbiological stability.[25]

The *Desmodium triquetrum Panchanga Yavakuta* used in the present study initially demonstrated the presence of *Aspergillus flavus* and *Penicillium* species. These organisms represent the natural microbial flora (commensals) found in the genus *Desmodium*, rather than pathogenic contamination responsible for aerobic infection. Following this initial observation, the *Yavakuta* was subjected to dry heat sterilization as a precautionary processing measure.

The dosage form intended for administration in the clinical study was *Kwatha* (decoction). The preparation involved heating the drug with distilled water under standardized conditions until bubbling was achieved, with the temperature reaching approximately 121 °C for 20 minutes. Microbiological examination of the prepared *Kwatha*, conducted after three days, revealed no detectable bacterial or fungal growth. This observation indicates that the standardized decoction process may serve as an important microbial control step and may contribute to maintaining the microbiological safety and quality of the preparation during the recommended period of consumption.

Despite the seasonal fluctuations in environmental temperature and humidity, serial microbiological assessments conducted at monthly intervals over a period of one year did not demonstrate microbial growth in the stored *Desmodium triquetrum* samples. The absence of microbial growth throughout the subsequent evaluations suggests that appropriate practices related to collection, drying, processing, packaging, and storage contributed to preservation of the microbiological quality and stability of the drug.

Similarly, consecutive monthly microbiological examinations of the *Kwatha* prepared from *Desmodium triquetrum* demonstrated satisfactory microbiological quality, with no detectable microbial growth in the evaluated samples. These findings further indicate that preparation of the drug in the form of *Kwatha*, following standardized processing procedures, may help minimize microbial proliferation in the final aqueous preparation.

Overall, the findings of the present study demonstrate that *Desmodium triquetrum Panchanga Kwatha* maintained satisfactory microbiological quality throughout the study period despite variations in ambient temperature and relative humidity. The application of baseline microbial diagnostic modalities facilitated systematic monitoring of the microbiological status of the preparation and demonstrated the importance of standardized processing and appropriate storage practices in maintaining its quality and stability.

The observations further support the use of *Desmodium triquetrum Panchanga Kwatha* as a dosage form in the management of *Kasa*, while emphasizing that appropriate hygienic practices, standardized preparation procedures, and suitable storage conditions are essential for ensuring microbiological safety. The study also highlights the relevance of incorporating microbial stability assessment into the quality evaluation of

Ayurvedic herbal preparations, particularly when the raw material is stored for an extended period under varying environmental conditions.

## CONCLUSION

The present microbial stability assessment demonstrates that *Desmodium triquetrum* (L.) DC. *Panchanga Kwatha* maintained satisfactory microbiological quality throughout the study period. Monthly microbiological examinations conducted under ambient environmental conditions, with temperatures ranging from 22.2 °C to 38 °C and relative humidity ranging from 34% to 80%, revealed no detectable bacterial or fungal growth in the subsequent samples following preparation of the *Kwatha*. Fungal filaments and fungal pathogens were not observed at any stage of the study.

*Aspergillus flavus* and *Penicillium* species were isolated at an early observation point from the *Yavakuta* form; however, they are commensal organisms associated with the plant species. Therefore, their detection in the raw plant material does not necessarily indicate the presence of pathogenic organisms or contamination responsible for producing aerobic infection. Importantly, these organisms were not detected in the subsequent samples of the *Kwatha* prepared by proper boiling. This observation indicates that appropriate boiling during preparation may contribute to reducing microbial contamination and maintaining the microbiological quality of the final *Kwatha* preparation.

Overall, the findings indicate that properly processed and stored *Desmodium triquetrum Panchanga Kwatha* remained microbiologically stable throughout the period of observation and was suitable for use under the conditions studied. The findings further support the traditional practice of storing the drug in *Yavakuta* form and preparing the *Kwatha* freshly before consumption, which may help minimize microbial proliferation in the aqueous preparation. Nevertheless, proper hygienic practices during preparation, handling, and storage, along with appropriate periodic microbiological evaluation, are essential for maintaining the microbiological quality and safety of the preparation.

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