ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

MICROBIAL PROFILE AND STABILITY ASSESSMENT OF *XANTHIUM STRUMARIUM* L. *YAVAKUTA* IN THE MANAGEMENT OF *MOOTRASHMARI* (UROLITHIASIS)

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Article info

Article History:
Received: 25-01-2026
Accepted: 26-02-2026
Published: 26-03-2026

KEYWORDS:
Microbial profile, Stability, *Xanthium strumarium, Mootrashmari, Urolithiasis.*

ABSTRACT

Ayurveda employs *Yavakuta* (coarse powder) as a fundamental dosage form for *Kwatha* preparation in systemic disorders such as *Mootrashmari* (urolithiasis). Although coarse powders possess relatively low moisture content compared to aqueous preparations, they remain vulnerable to environmental microbial contamination during collection, processing, and storage. *Xanthium strumarium* L. has been traditionally reported for its use in kidney-related disorders and is regionally utilized as a substitute for *Gokshura* in certain parts of Punjab and Sindh due to its *Mutrala* (diuretic) properties and local availability. **Materials and Methods**: The microbiological profile of whole-plant *Yavakuta* of *Xanthium strumarium* was evaluated under simulated storage conditions from April 2024 to April 2025. Samples were periodically analyzed using Gram staining, 10% KOH wet mount, aerobic bacterial culture, and fungal culture techniques under varying temperature (28–42°C) and relative humidity (15–96%) conditions. **Results:** Most samples demonstrated absence of microbial growth. Intermittent isolation of *Citrobacter* species was observed during certain intervals, which are commonly reported as plant-associated microorganisms. No persistent bacterial contamination was detected, and no fungal growth was observed throughout the study period, even under high humidity conditions. **Conclusion:** The *Yavakuta* form of *Xanthium strumarium* exhibited satisfactory microbial stability under fluctuating environmental conditions. These findings support its safe storage and utilization in coarse powder form, provided that appropriate hygienic handling and periodic microbial monitoring are maintained.

INTRODUCTION

Yavakuta (coarse powder) is an important pharmaceutical form in Ayurveda and serves as the primary raw material for *Kwatha* preparation under *Panchavidha Kashaya Kalpana.* [1] The microbial quality of *Yavakuta* directly influences the safety, efficacy, and shelf life of the final formulation. Although coarse powders contain relatively low moisture compared to aqueous preparations, they remain susceptible to environmental microbial contamination during collection, shade-drying, pulverization, handling, and storage.

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https://doi.org/10.47070/ijapr.v14i3.4039

Xanthium strumarium L. is traditionally reported for its use in kidney-related disorders and is known for its *Mutrala* (diuretic) properties. In certain regions such as Punjab and Sindh, it is used as a substitute for *Gokshura* due to similarity in therapeutic indications and local availability. [2] Since whole-plant (*Panchanga*) material is utilized in *Yavakuta* preparation, the possibility of residual plant-associated microflora cannot be excluded. Coastal climatic conditions such as those in Jamnagar, characterized by fluctuating temperature and humidity, may favour microbial survival and persistence. Therefore, systematic evaluation of microbial stability under such environmental conditions is essential to ensure safety and quality.

Although *Yavakuta* possesses comparatively lower moisture than aqueous formulations, it remains vulnerable to environmental microbial contamination

during stages such as collection, shade-drying, pulverization, handling, and storage. Contamination of crude powders can adversely affect their quality, safety, and shelf life. Therefore, systematic assessment of microbial load is crucial to ensure quality control and safe usage.

Microbial survival and growth are largely influenced by environmental factors, particularly temperature and relative humidity. Most bacteria and fungi thrive within a temperature range of 20–40°C and relative humidity levels of 60–90%. Consequently, periodic evaluation of the microbial profile under varying storage conditions is necessary to confirm the microbiological stability of *Yavakuta*.

AIM

To assess the microbial profile and storage stability of *Xanthium strumarium* L. *Yavakuta* (coarse powder) at different time intervals under varying climatic conditions of temperature and relative humidity.

OBJECTIVE

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

MATERIALS AND METHODS

A stability assessment was undertaken to examine the microbial profile of *Xanthium strumarium* L. *Yavakuta* from the time of preparation until completion of the study period. The raw plant material was obtained from authenticated sources and carefully processed to ensure quality. Extraneous matter was removed, the material was thoroughly cleaned, shade dried, and prepared in accordance with classical Ayurvedic pharmaceutical principles using potable water and standardized procedures. The final *Yavakuta* was packed in sterile containers and stored at room temperature. Microbiological evaluation was conducted periodically over a twelve-month period, corresponding to the intended duration of drug usage. All microbiological investigations were performed under controlled laboratory conditions at the Microbiology Laboratory, I.T.R.A., Jamnagar, to detect any bacterial or fungal contamination. The first microbial assessment was carried out on the 115th day after preparation, prior to patient enrolment in the clinical study, and subsequent analyses were conducted at scheduled as well as random intervals across different seasonal variations. Furthermore, the stability of whole-plant powder prepared on 10/12/2023 was monitored from April 2024 to April 2025 under simulated storage conditions, during which temperature ranged from 28–42 °C and relative humidity varied between 15–96%.

Drug Material

Whole plants of *Xanthium strumarium* L. were collected from the vicinity of Jamnagar, Gujarat (approximately 22.4707° N, 70.0577° E), following Good Collection Practices during Sharad Ritu. Botanical identification was carried out based on detailed macroscopic and organoleptic characteristics with reference to standard regional floras and authenticated sources. Only mature plants were selected for processing. After thorough washing with running potable water to remove soil and other extraneous matter, the material was shade dried. The Panchanga was then coarsely powdered using sieve size 40 to prepare *Yavakuta*. The prepared coarse powder was stored in airtight containers under suitable conditions and was subsequently used for *Kwatha* preparation in the clinical study.

MICROBIAL PROFILE

Microbial contamination of the *Yavakuta churna* was assessed using two standard microbiological approaches to detect both bacterial and fungal contamination: [3]

1. Smear Examination

- **10% KOH Preparation**
- **Gram's Stain**

2. Culture Study

- **Fungal culture**
- **Aerobic bacterial culture**

SMEAR EXAMINATION

Procedure For 10% Koh Preparation:

1. Take Potassium Hydroxides pellets (of HiMedia Lab. Pvt. Ltd.) in distilled water to prepare 10% of the same in clean glass tube & mix well
2. Take clean grease free glass slide
3. Put a-drop of decoction of both the plant separately and add freshly prepared10% KOH than cover with grease free cover glass
4. Allow it to react for 15-20 minutes to remove extra debris other than fungal particles
5. Observe under high power (40x) lens
6. Report as per finding

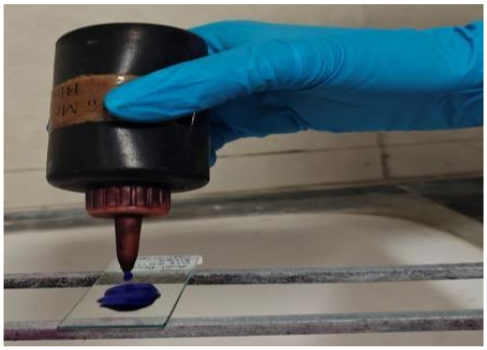

Figure 1: Smear staining Procedure

Gram's Stain

Procedure for Gram's Stain:

1. Take clean grease free glass slide to prepare dry equal thick preparation (i.e., smear of *Yavakuta* preparation)
2. 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)
3. Cover fixed prepared smear with Gram's crystal violet solution and allow to remain for mentioned time as per kit procedure
4. Washed off smear to remove excessive reagent with tap water
5. Cover the smear with Gram's Iodine solution and allow to remain for mentioned time as per kit procedure
6. Washed off smear to remove excessive reagent with tap water
7. 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
8. Cover smear with Safranin solution and allow to remain for mentioned time as per kit procedure
9. Washed off smear to remove excessive reagent with tap water
10. Blot and allow to dry smear
11. Examine under oil immersion lens and report as per findings

Figure 2: Stained Smear Procedure

CULTURE STUDY

Fungal Culture

The sample was inoculated on Sabouraud Dextrose Agar using sterile techniques. The inoculated media were incubated at the required temperature for the specified duration and observed for fungal growth.

Procedure For Fungal Culture:

1. In the clinical microbiology laboratory, culture method was employed for isolation of organisms (The lawn / streak culture method is routinely employed)
2. Sabouraud Dextrose Agar Base (SDA) selected as solid media for inoculation purpose
3. Dry selective solid media in Hot Air Oven before specimen inoculation, allowed to cool dried medium before specimen inoculation
4. Inoculate *Yavakuta churna* by Sterile cotton swab or by Nichrome wire (24 5.W.G. size) loop [First sterile loop in Bunsen burner oxidase flames blue flame and allow it to cool than loop was charged with *Yavakuta churna* and cultured. One loopful of the *Yavakuta churna* was transferred onto the surface of well dried culture media]
5. After inoculation / streaking process, Incubate inoculated medium kept 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
6. After selected incubation period, examined the growth by naked 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.

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

Aerobic Culture

Respected materials 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 ºC

Use of media: For selective cultivation of pathogenic bacteria.

Procedure For Aerobic Culture:

1. In the clinical microbiology laboratory culture method are employed for isolation of organisms (The streak culture method is routinely employed)
2. MacConkey Agar (MA) was selected as solid media for inoculation purpose
3. Dry selective solid media in Hot Air Oven before specimen inoculation, allow to cool dried medium before specimen inoculation
4. Inoculate the coarse powder 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 was charged with *Yavakuta Churna* to be culture.
5. After streaking process Incubate inoculated medium in inverted position at 37°C for 18- 24 hours in Incubator under aerobic or 10% CO2 atmosphere
6. After selected incubation period, examined the 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 isolate

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

Figure 5: Preparation with MacConkey Agar (MA)

RESULTS

The evaluation included Gram's staining, 10% KOH wet mount, aerobic bacterial culture, and fungal culture. Smear examination and culture findings demonstrated absence of bacterial and fungal contamination in both drugs throughout the study period.

The observations of smear examination and culture studies at various time intervals are presented in the respective tables. Throughout the study period, no bacterial or fungal growth was detected in the sample of *Xanthium strumarium Yavakuta* (Coarse powder). [Table 1]

Drug preparation date: 10/12/2023

Table 1: Observation of *Xanthium strumarium Yavakuta*

S.No	Date & Days of investigations After preparation of the sample	Temperature (˚C)	Humidity (%)	Observation of Sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH	Fungal culture
1	03/04/2024 115th day	39˚C	32%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
2	08/05/2024 150th day	42 ˚C	44%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
3	20/06/2024 193rd day	40 ˚C	60%	Micro-organisms not seen	Citrobacter species isolated*	Fungal filaments not seen	No fungal pathogen

4	23/07/2024 226th day	30 ˚C	85%	Micro-organisms not seen	Citrobacter species isolated*	Fungal filaments not seen	No fungal pathogen
5	03/09/2024 268th day	31 ˚C	96%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
6	03/10/2024 298th day	35 ˚C	60%	Presence of capsulated gram-negative rods arranged singly	Citrobacter species isolated*	Fungal filaments not seen	No fungal pathogen
7	09/10/2024 304th day	38 ˚C	54%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
8	11/11/2024 337th day	28 ˚C	54%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
9	9/12/2024 365th day	28 ˚C	76%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
10	16/01/2025 403rd day	35 ˚C	92%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
11	17/02/2025 435th day	38 ˚C	15%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
12	17/03/2025 463rd day	42 ˚C	91%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen
13	24/04/2025 499th day	37 ˚C	84%	Micro-organisms not seen	Absence of microorganisms	Fungal filaments not seen	No fungal pathogen

*Isolated species found as a commensal of *Xanthium strumarium* for plant growth
Isolated organism not considered as a contamination

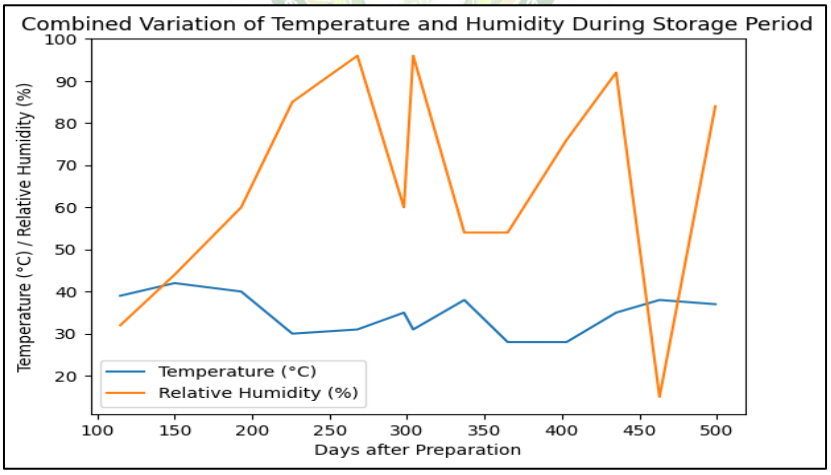

Figure 6: Temperature and Relative Humidity Profile Under Simulated Storage Conditions

DISCUSSION

The present investigation specifically assessed the microbiological stability of *Xanthium strumarium Yavakuta* during prolonged storage under variable coastal environmental conditions. Although coarse powders inherently possess lower moisture content than liquid formulations, they remain vulnerable to environmental exposure during post-harvest processing and storage. Temperature fluctuations (28–42 °C) and wide variations in relative humidity (15–96%) observed during the study period represent conditions commonly encountered in tropical coastal regions and may influence microbial persistence. Standard microbiological principles[4] emphasize that environmental exposure during post-harvest handling and storage can introduce transient microflora into crude plant powders.

During the evaluation period, intermittent isolation of *Citrobacter* species was noted. Members of this genus are frequently encountered in soil and plant ecosystems and are recognized as components of environmental microflora.[5] Considering that whole-plant (*Panchanga*) material was processed into *Yavakuta*, the occasional detection of such organisms is likely attributable to inherent plant-associated microbiota rather than active post-processing contamination. Importantly, no progressive increase in microbial load was observed across subsequent intervals, supporting the absence of sustained microbial proliferation. The affected pouches were discarded immediately in accordance with laboratory quality control protocols.

A significant observation of this study was the consistent absence of fungal growth, even during periods of high relative humidity. Since fungal contamination is closely linked with increased moisture and water activity, the findings reflect adequate shade-drying and effective moisture control during preparation and storage. This highlights the importance of controlled drying practices in maintaining the microbiological integrity of crude plant powders.

Given the traditional use of *Xanthium strumarium* in renal disorders and its regional application in formulations where it serves as a substitute for *Gokshura*, ensuring its microbiological safety in *Yavakuta* form is essential. The findings demonstrate that, under appropriate handling and storage conditions, the coarse powder remains microbiologically stable even in environmentally challenging settings.

CONCLUSION

The *Yavakuta* form of *Xanthium strumarium* demonstrated stable microbiological quality during extended storage under fluctuating coastal climatic conditions. The occasional isolation of environmental bacteria was transient and non-persistent, and no fungal contamination was detected. Although commensal microorganisms were present, these represent common environmental flora and are non-pathogenic in nature; hence, their presence does not compromise the safety of the formulation for consumption. These findings indicate that properly processed and stored *Xanthium strumarium Yavakuta* is microbiologically safe for consumption, provided that hygienic practices and periodic microbial assessments are consistently maintained.

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Cite this article as:
Payal Purohit, M.S. Cholera, Sreebala G, Brijen Ramavat, Bhupesh R. Patel. Microbial Profile and Stability Assessment of Xanthium Strumarium L. Yavakuta in the Management of Mootrashmari (Urolithiasis). International Journal of Ayurveda and Pharma Research. 2026;14(3):217-222.
https://doi.org/10.47070/ijapr.v14i3.4039
Source of support: Nil, Conflict of interest: None Declared

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