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"STABILITY AND MICROBIAL PROFILING OF *AGNIDAMANI* (*SOLANUM TRILOBATUM* LINN.) *YAVAKUTA* AND EVALUATION OF *KWATHA* PREPARED THEREFROM IN THE MANAGEMENT OF *KASA* (COUGH)"

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Abstract

Background: *Agnidamani* (*Solanum trilobatum* Linn.), belonging to the family Solanaceae, is traditionally indicated in the management of *Kasa* (cough), bronchial asthma, and other respiratory disorders. The whole plant (*Panchanga*) is used therapeutically in the form of *Kwatha*. As *Kwatha* is a freshly prepared aqueous extract with high moisture content and absence of preservatives, its microbial quality may be influenced by environmental conditions such as temperature and relative humidity. **Aim:** To evaluate the stability and microbial quality of *Agnidamani* (*Solanum trilobatum* Linn.) *Kwatha* prepared from *Yavakuta* stored under specified temperature and humidity conditions at different time intervals. **Materials and Methods:** Whole plants of *Solanum trilobatum* L. were collected from the vicinity of Chengalpattu, Tamil Nadu, during May 2025, cleaned, shade dried, and coarsely powdered to obtain *Yavakuta*. Preparation of the *Kwatha* involved heating the drug with distilled water (i.e. decoction form) up to bubbling under standardized conditions, temperature reaching approximately 121°C for 20 minutes. Microbiological evaluation was carried out at the Microbiology Laboratory, I.T.R.A., Jamnagar. Smear examination by Gram's stain and 10% KOH preparation,

along with aerobic bacterial culture and fungal culture, was performed periodically from July 2025 to June 2026 under ambient conditions. Temperature during the study period ranged from 22°C to 38°C and relative humidity from 34% to 80%. **Results:** Initial microbiological evaluation of *Agnidamani Panchanga Yavakuta* revealed many Gram-negative pleomorphic rods arranged singly, and culture studies identified Pseudomonas species and Enterobacter species. These organisms were considered plant-associated commensals and not contamination. Subsequent evaluation of freshly prepared *Agnidamani Kwatha* showed no microorganisms on Gram's staining, no bacterial growth on aerobic culture, no fungal filaments on 10% KOH examination, and no fungal pathogen on fungal culture. These findings remained consistent throughout the monthly evaluations during the twelve-month study period. **Conclusion:** The freshly prepared *Agnidamani* (*Solanum trilobatum* Linn.) *Panchanga Kwatha* showed complete absence of bacterial and fungal growth after processing throughout the study period. The findings indicate that the preparation method effectively eliminates viable microbial contaminants and supports the microbiological quality and safety of *Agnidamani Kwatha* when prepared under appropriate conditions.

Keywords: *Agnidamani*, *Solanum trilobatum* Linn., *Yavakuta, Kwatha, Kasa*, Microbial profile, Stability, Gram's stain, Fungal culture.

INTRODUCTION

Agnidamani (*Solanum trilobatum* Linn.), a medicinal plant belonging to the family *Solanaceae*, is traditionally indicated in the management of *Kasa* (cough), bronchial asthma, and other respiratory disorders.[i] The botanical identity of *Agnidamani* as *Solanum trilobatum* Linn. has been established by Acharya Bapalal Vaidya in Adarsha Nighantu based on the description of *Agnidamani* mentioned in Raj Nighantu.[ii] *Kwatha* (decoction) is one of the most important and widely used dosage forms in Ayurveda. It is prepared by boiling coarse powder of medicinal plants with a specified quantity of water until reduction to the desired volume is achieved.[iii] This process facilitates the extraction of water-soluble phytoconstituents, contributing to its therapeutic efficacy and bioavailability.

The whole plant (*Panchanga*) of *Agnidamani* is used therapeutically in the form of *Kwatha*; however, the presence of naturally occurring plant-associated microflora may influence the microbial quality of the raw material and the final preparation. As *Kwatha* is a freshly prepared Ayurvedic dosage form consisting of an aqueous extract of herbal drugs, its high moisture content and absence of preservatives may influence its microbial quality. Furthermore, variations in environmental conditions such as temperature and relative humidity may affect microbial survival and proliferation during handling and storage. Therefore, periodic evaluation of microbial quality over a defined study period is essential to assess the safety, quality, and therapeutic reliability of *Agnidamani Kwatha.*

Microbial profiling involves the estimation of total microbial load and detection of specified pathogenic microorganisms using standard microbiological techniques. Stability studies further provide information regarding the maintenance of microbial quality under defined storage conditions, thereby generating scientific evidence regarding the safety and suitability of the formulation.

AIM

To evaluate the stability and microbial quality of *Agnidamani* (*Solanum trilobatum* Linn.) *Kwatha* prepared from *Yavakuta* stored under specified temperature and humidity conditions at different time intervals.

OBJECTIVE

To ensure compliance with standardization and quality control parameters, the microbial profile of *Agnidamani* (*Solanum trilobatum* Linn.) *Kwatha* has been evaluated throughout the study period to assess its quality and stability.

MATERIAL AND METHODS

The raw plant materials were procured from authenticated sources, cleaned, dried, and processed according to classical Ayurvedic pharmaceutical procedures to prepare the *Yavakuta*. The standardized *Yavakuta* was administered during the initial visit, while *Agnidamani Kwatha* was prepared fresh from the same *Yavakuta* before each subsequent administration using standardized preparation methods. Microbiological evaluation was performed periodically throughout the twelve-month study period to ensure the microbial quality of the formulation during its intended use. Periodic microbiological evaluation of freshly prepared *Kwatha* samples, was carried out at the Microbiology Laboratory, I.T.R.A., Jamnagar, to detect bacterial and fungal contamination and to ensure the microbiological quality and safety of the formulation throughout the study period. The initial microbiological evaluation was carried out on the 07/07/2025, before the commencement of patient enrolment. Subsequent evaluations were performed periodically at scheduled intervals across different seasons. The microbial quality of the *Kwatha* was monitored from July 2025 to June 2026 under ambient conditions.

Drug Material

Whole plants of *Solanum trilobatum* L. (*Agnidamani*) were collected from the vicinity of Chengalpattu, Tamil Nadu (Latitude: 12° 41′ 38.1558″ N; Longitude: 79° 58′ 32.3882″ E) during May 2025, following Good Collection Practices. Botanical authentication was performed based on detailed macroscopic and organoleptic characteristics with reference to standard regional floras and authenticated botanical literature. Only healthy, mature plants were selected for the study. The collected material was thoroughly washed with running potable water to remove adhering soil and other extraneous matter and subsequently shade dried under appropriate conditions. The dried whole plant material was coarsely powdered using sieve No. 40 to obtain *Yavakuta*. The prepared coarse powder was stored in clean, airtight containers under suitable storage conditions until further use for *Kwatha* preparation and subsequent microbiological evaluation.

Preparation of *Kwatha* of *Solanum trilobatum* L.

The *Kwatha* of *Solanum trilobatum* L. was prepared according to the classical method.[iii] Preparation of the *Kwatha* involved heating the drug with distilled water (i.e. decoction form) up to bubbling under standardized

conditions, temperature reaching approximately 121°C for 20 minutes. The freshly prepared *Kwatha* was used for microbiological analysis.

MICROBIAL PROFILE

Microbial contamination of the *Solanum trilobatum* L. *Kwatha* was evaluated using two standard microbiological methods for the detection of bacterial and fungal contaminants.[iv]

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 the plant and add freshly prepared 10% 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.

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 *Kwatha* 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.

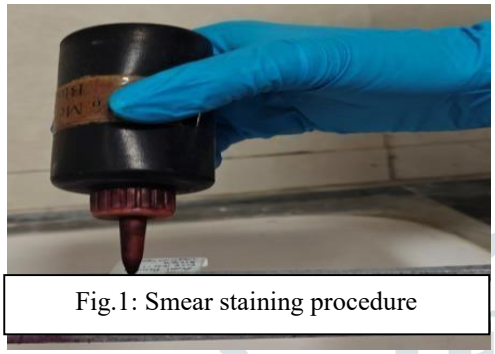

Fig.1: Smear staining procedure

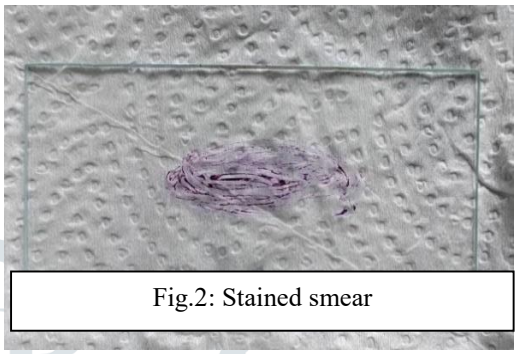

Fig.2: Stained smear

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 *Kwatha* 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 *Kwatha* and cultured. One loopful of the *Kwatha* 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.

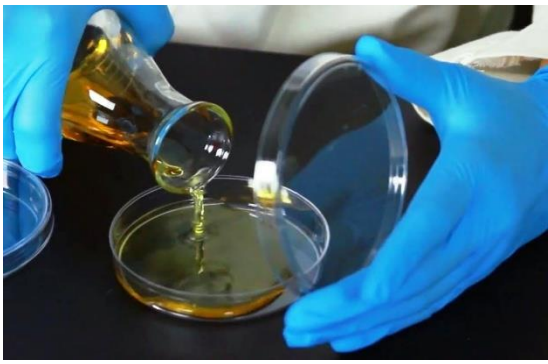

Fig.3: Fungal culture media preparation with Sabouraud Dextrose Agar Base (SDA)

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 °C

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 *Kwatha* 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 *Kwatha* to 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 biochemical reactions and different related staining procedures. After that report isolate.

Fig.4: Preparation with MacConkey Agar (MA)

RESULTS

The evaluation included Gram staining, 10% KOH examination, aerobic bacterial culture, and fungal culture to assess the microbial quality of *Solanum trilobatum* L. *Kwatha*. Smear examination and culture findings demonstrated the absence of bacterial and fungal contamination in the formulation throughout the study period.

The observations from smear examination and culture studies at different time intervals are presented in the below table. Throughout the study period, no bacterial or fungal growth was detected in the samples of *Solanum trilobatum* L. *Kwatha*, indicating that the formulation remained microbiologically stable and free from microbial contamination upon preparation. (Table 1)

Microbiological Evaluation Commenced On: 07/07/2025

Table 1: Showing results of microbial profile of *Solanum trilobatum* L. *Kwatha*.

Sr. No	Date of investigation after collection of raw drug	Temp. (˚C)/ Humidity (%)	Name of the sample	Observations of sample			
				Gram's Stain	Aerobic culture	10% KOH	Fungal culture
1	07/07/2025 Drug provided in *Yavakuta* form	29˚C / 78%	*Agnidamani Panchanga Yavakuta*	Presence of many gram-negative pleomorphic rods arranged singly*	*Peudomonas* species & *Enterobacter* species isolated*	Fungal filaments not seen	No fungal pathogen isolated
2	10/07/2025 Drug provided in *Kwatha* form boiled at 121 ˚C for 20 mins	29˚C / 78%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
3	11/08/2025	28˚C / 80%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
4	15/09/2025	28˚C / 77%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
5	16/10/2025	29˚C / 56%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated

6	10/11/2025	26˚C / 47%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
7	18/12/2025	22˚C / 48%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
8	19/01/2026	28˚C / 52%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
9	17/02/2026	30˚C / 47%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
10	16/03/2026	33˚C / 34%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
11	15/04/2026	34˚C / 40%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
12	19/05/2026	38˚C / 50%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated
13	15/06/2026	33˚C / 69%	*Agnidamani Kwatha*	Micro-organisms not Seen	No organisms isolated	Fungal filaments not seen	No fungal pathogen isolated

*Isolated species found as a commensals of *Solanum trilobatum* L. for plant growth. Isolated organism not considered as a contamination.

DISCUSSION

The present microbiological study was undertaken to evaluate the microbiological quality of *Agnidamani Panchanga Kwatha* (*Solanum trilobatum* Linn.) following its preparation. Microbiological evaluations were conducted at monthly intervals under ambient environmental conditions, with recorded temperatures ranging from 22°C to 38°C and relative humidity between 34% and 80%. During the initial microbiological assessment, the *Yavakuta* (coarse powder) of *Solanum trilobatum Panchanga* revealed Gram-negative pleomorphic rods, and culture studies identified *Pseudomonas* and *Enterobacter* species. Members of these genera are widely recognized as plant-associated bacteria, exhibiting ecological relationships that range from mutualism to pathogenesis.[v] Therefore, their detection in the raw plant material does not necessarily indicate pathogenic organisms as a contamination responsible for producing HAI's infection. Considering these

organisms as commensals, the decoction process was subsequently carried out to ensure the standard quality and microbial safety of the study drug.

Subsequent microbiological evaluation was performed on freshly prepared *Kwatha* samples.

Preparation of the *Kwatha* involved heating the drug with distilled water (i.e. decoction form) up to bubbling under standardized conditions, temperature reaching approximately 121°C for 20 minutes. Following preparation, the samples were subjected to smear examination and microbial culture. No detectable bacterial or fungal growth was observed in any of the monthly samples examined throughout the study period. These findings indicate that the freshly prepared *Agnidamani* (*Solanum trilobatum* Linn.) *Panchanga Kwatha* complied with acceptable microbiological quality standards under the prevailing environmental conditions during the study period.

CONCLUSION

The present study evaluated the microbiological quality of *Agnidamani* (*Solanum trilobatum* Linn.) *Panchanga Kwatha* through Gram's staining, aerobic bacterial culture, 10% KOH and fungal culture. The raw drug (*Yavakuta*) showed the presence of Gram-negative pleomorphic rods, and culture studies revealed *Pseudomonas* and *Enterobacter* species, which are recognized as plant-associated microorganisms. However, the freshly prepared *Kwatha* samples, evaluated at monthly intervals, showed complete absence of bacterial and fungal growth after processing.

The findings indicate that the preparation method of *Agnidamani* (*Solanum trilobatum* L.) *Panchanga Kwatha* effectively eliminates viable microbial contaminants, resulting in a microbiologically safe formulation. The study supports the quality and safety of the prepared *Kwatha* for therapeutic use when prepared under appropriate conditions.

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