Stability study of Kumari (Aloe vera [L.] burm.) Swarasa (juice) with respect to baseline microbial diagnostic modalities

Pravin Jawanjal, M. S. Cholera, Prashant Bedarkar, B. J. Patgiri

Abstract:

BACKGROUND: Swarasa, one of the most popular Kalpana among five basic Kalpana, is widely used therapeutically as well as pharmaceutically. A clear description of Swarasa is available in all Samhitas regarding its preparation, and utility.

AIMS: This study aims to carry out the stability of Kumari Swarasa with respect to its microbial profile.

MATERIALS AND METHODS: Sample of Kumari Swarasa was prepared and studied to check microbial contamination at regular intervals.

RESULTS: Five samples were subjected to the microbiological study from the date of the preparation to the date of last microbiological study. No contaminations were found in the microbiological study in the range of minimum of one day and a maximum of three days after preparation of sample.

DISCUSSION: Hence, the present study was carried out to observe the stability study of Kumari Swarasa with respect to microbial contamination of samples prepared and preserved in different climatic and temperature conditions. Thus, a baseline microbial profile was studied at regular intervals. At the end of the study, it was found that shelf life of Aloe vera juice was varied as per humidity and temperature.

CONCLUSION: The microbiological study of Kumari Swarasa shows its stability in the range of a minimum of one day and maximum of three days after the preparation of the sample.

Keywords: Aloe Vera, Kumari, Swarasa, Stability
stimulation of synthesis or release of insulin from beta-cells of the pancreas. Dried sap of *aloe vera* showed significant hypoglycemic effect clinically as well as experimentally.⁴⁴ *A. vera* gel (200 mg/kg) possesses significant antidiabetic and cardioprotective activity and maintains superoxide dismutase and catalase activity up to normal and increases glutathione four times in diabetic rats.⁴⁵ The amendment of Rule No. 161-B of Drugs and Cosmetic Act 1940, specify the maximum shelf life or date of expiry of different dosage form of ayurvedic drugs. The shelf life of fresh prepared *Swarasa* is not mentioned in the Gazette of India.⁶

The drug was prepared in RS and BK dept. I. P. G. T and R. A., Jamnagar of Gujarat Ayurved University, Jamnagar. No preservative was added to the test drug.

**Aim**

This study aims to study the stability of the finished product and to check microbial contamination in the finished product at different time intervals - at different climatic conditions, temperature, and humidity setups.

**Materials and Methods**

**Drug preparation**

*Kumari Swarasa* was prepared by according to “Sharangadhara Samhita.”⁷ And studied to check microbial contamination at regular intervals. The microbiological study has been carried out in Microbiology Laboratory, IPGT and RA, Jamnagar.

**Drug material**

The drug was prepared in RS and BK Department IPGT and RA, Jamnagar of Gujarat Ayurved University, Jamnagar.

**Storage**

The finished product was stored in airtight food-grade, plastic containers, stored in the open light area in the department at room temperature. A 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% KOH preparation
   b. Gram’s stain.
2. Culture study
   a. Fungal culture
   b. Aerobic culture.

The details of the procedures followed are given below.

**Smear examination**

**Wet mount/10% KOH preparation**

- **Aim:** To rule out any mycological findings.
- **Specimen:** *Kumari Swarasa.*

**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 counterstain effect was 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.⁸

- **Aim:** To rule out any bacteriological findings
- **Specimen:** *Kumari Swarasa.*
Fungal culture method
Respected materials collected with a sterile cotton swab for inoculation purpose on selected fungal culture media (i.e., an artificial preparation).
- Name of media: Sabouraud dextrose agar base, modified (dextrose agar base, emmons)
- Company: HIMEDIA Laboratories Pvt. Ltd.
- Required time duration: 5–7 days
- Required temperature: 37°C
- Use of media: For selective cultivation of pathogenic fungi.

Aerobic culture method
Respected materials collected with a sterile cotton swab for inoculation purpose on selected aerobic culture media (i.e., an artificial preparation).
- Name of media: Mac Conkey Agar and Columbia blood agar
- Company: HIMEDIA Laboratories Pvt. Ltd.
- Required time duration: 24–48 h
- Required temperature: 37°C
- Use of media: For selective cultivation of pathogenic bacteria.
Observations and Results

Every time, sample (in which drug preserved) was subjected to the microbiological study from the date of the preparation to the date of the last microbiological study.

Results are shown in Table 1.

Discussion

The present study was carried out to observe *Kumari Swarasa* with respect to microbial contamination of samples prepared and preserved in different climatic and temperature conditions. Thus, a baseline microbial profile was studied at regular intervals. At the end of study, it was found that sample showed the presence of microbes in batch one on 3rd day from date of preparation, in batch two on 2nd day from the date preparation, in batch 3 on 1st day, in batch five on 3rd day, and in batch six on 3rd day from date of preparation were found. The main factors affecting the

| Days of investigations after preparation of the sample at | Date of sample given | Gram’s stain | Aerobic culture | Wet mount/10% KOH preparation | Fungal culture |
|---------------------------------------------------------|----------------------|--------------|-----------------|-----------------------------|---------------|
| Batch-1 Day-1                                           | August 23, 2018      | Microorganisms Not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | August 24, 2018      | Microorganisms Not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-2 Day-1                                           | August 27, 2018      | Microorganisms Not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | August 28, 2018      | The smear shows presence of many capsulated Gram-negative rods arranged singly | Escherichia coli isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-3 Day-1                                           | October 11, 2018     | The smear shows presence of many capsulated Gram-negative rods arranged singly | Escherichia coli isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-4 Day-1                                           | April 24, 2019       | Microorganisms Not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | April 25, 2019       | Microorganisms Not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Day-3                                                   | April 26, 2019       | The smear shows presence of many capsulated Gram-negative rods arranged singly | Escherichia coli isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-5 Day-1                                           | July 1, 2019         | Microorganisms Not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | July 2, 2019         | Microorganisms Not seen | No organisms isolated | Fungal filaments not seen | No fungal pathogen isolated |
| Day-3                                                   | July 3, 2019         | The smear shows presence of many capsulated Gram-negative rods arranged singly | Escherichia coli isolated | Fungal filaments not seen | No fungal pathogen isolated |

Shelf life are the derivation of the drug, dosage forms, environmental factors (humidity, temperature, and light), microbial contamination, storage conditions, and packaging system.

Stability is usually expressed in terms of shelf life, which is the time period from when
Table 2: Observations of *Kumari swarasa* preserved at room temperature

| Days of investigations after preparation of the sample at | Date of sample given | Temperature | Humidity (%) | Observations of sample |
|----------------------------------------------------------|-----------------------|-------------|--------------|------------------------|
|                                                          |                       |             |              | Wet mount/10% KOH preparation | Fungal culture |
| Batch-1                                                  |                       |             |              | Fungal filaments not seen | No fungal pathogen isolated |
| Day-1                                                   | August 23, 2018       | 28°C        | 80           | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | August 24, 2018       | 28°C        | 78           | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-2                                                  |                       |             |              | Fungal filaments not seen | No fungal pathogen isolated |
| Day-1                                                   | August 27, 2018       | 27°C        | 77           | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | August 28, 2018       | 29°C        | 76           | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-3                                                  |                       |             |              | Fungal filaments not seen | No fungal pathogen isolated |
| Day-1                                                   | October 11, 2018      | 34°C        | 76           | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-4                                                  |                       |             |              | Fungal filaments not seen | No fungal pathogen isolated |
| Day-1                                                   | April 24, 2019        | 35°C        | 29           | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | April 25, 2019        | 35°C        | 32           | Fungal filaments not seen | No fungal pathogen isolated |
| Day-3                                                   | April 26, 2019        | 37°C        | 26           | Fungal filaments not seen | No fungal pathogen isolated |
| Batch-5                                                  |                       |             |              | Fungal filaments not seen | No fungal pathogen isolated |
| Day-1                                                   | July 1, 2019          | 32°C        | 59           | Fungal filaments not seen | No fungal pathogen isolated |
| Day-2                                                   | July 2, 2019          | 33°C        | 57           | Fungal filaments not seen | No fungal pathogen isolated |
| Day-3                                                   | July 3, 2019          | 33°C        | 59           | Fungal filaments not seen | No fungal pathogen isolated |

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. Shelf life according to *Yogaratnakara* is 3 h (one *Prahar*).[^9] In batch three, it was found that contamination on 1st day from the date of preparation which was same as acharya *Yogaratnakara* opinion, but in batch four and five contamination was found on 3rd day. It revealed that shelf life of *A. vera* juice was varied as per humidity and temperature [Tables 1 and 2].

**Conclusion**

The microbiological study of *Kumari Swarasa* shows its stability in the range of a minimum of one day and maximum of three days after the preparation of the sample.

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Conflicts of interest
There are no conflicts of interest.

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