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MICROBIOLOGICAL QUALITY ASSESSMENT OF AYURVEDIC FORMULATION AMLOKIRASAYANA AND SARIBADYARISTA

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ABSTRACT

Ayurvedic herbal formulations are widely used for the prevention and treatment of various health conditions. However, due to their natural origin and complex preparation processes, these products may be susceptible to microbial contamination during manufacturing, storage, or handling. Ensuring the microbiological quality of such formulations is essential to guarantee their safety, efficacy, and compliance with established pharmacopeial standards. The present study aimed to evaluate the microbiological quality of two commonly used Ayurvedic formulations, Amlokirasayana and Saribadyarista, by determining their total viable bacterial count and total yeast and mold count. A total of twenty samples were analyzed in this study, consisting of ten samples of Amlokirasayana (AMR-1 to AMR-10) and ten samples of Saribadyarista (SBR-1 to SBR-10). Microbiological analysis was conducted using standard plate count methods to determine the total aerobic microbial count (TAMC) and total yeast and mold count (TYMC). Serial dilution techniques were

employed, and appropriate culture media were used to facilitate the growth of bacteria, yeast, and molds. The microbial colonies formed were counted and expressed as colony-forming units per milliliter (CFU/mL). The obtained results were then compared with acceptable microbiological limits for herbal medicinal products, where the permissible limits are $\leq 1 \times 10^5$ CFU/mL for total aerobic bacterial count and $\leq 1 \times 10^3$ CFU/mL for total yeast and mold count. The results revealed that the total viable bacterial count in Amlokirasayana samples ranged from 1×10^1 to 1×10^4 CFU/mL, while the total yeast and mold count ranged from 1×10^1 to 1×10^3 CFU/mL. Similarly, the Saribadyarista samples showed total viable bacterial counts ranging from 1×10^1 to 1×10^3 CFU/mL and yeast and mold counts ranging from 1×10^1 to 1×10^3 CFU/mL. In all tested samples, the microbial counts were found to be within the acceptable limits specified for herbal medicinal preparations. The findings of this study indicate that both Amlokirasayana and Saribadyarista formulations exhibit satisfactory microbiological quality. The relatively low microbial counts observed in the samples suggest that appropriate manufacturing practices, hygienic handling, and adequate storage conditions were maintained during the preparation and distribution of these products. Additionally, the inherent antimicrobial properties of certain herbal ingredients present in these formulations may contribute to limiting microbial growth. In conclusion, the microbiological quality assessment demonstrated that the tested samples of Amlokirasayana and Saribadyarista comply with acceptable microbial standards and are considered microbiologically safe for consumption. Continuous monitoring of microbial contamination in herbal formulations is essential to ensure product safety and maintain public confidence in traditional medicinal systems. This study highlights the importance of routine microbiological evaluation as part of quality control measures for Ayurvedic formulations.

KEYWORDS: Ayurvedic formulation; Amlokirasayana: and Saribadyarista: Total viable bacterial count; CFU/ml; Microbial contamination.

1. INTRODUCTION

1.1. Background

Traditional systems of medicine have been practiced for centuries and continue to play a significant role in global healthcare. According to the World Health Organization (WHO): a substantial proportion of the population in developing countries relies on traditional medicine for primary healthcare needs. Among various traditional medical systems, Ayurveda, originating in the Indian subcontinent, is one of the oldest holistic systems of medicine, with

documented use spanning over 3,000 years (Sharma, 2001). Ayurveda emphasizes the balance between body, mind, and environment and employs natural resources such as medicinal plants, minerals, and animal products for disease prevention and health promotion. Ayurvedic formulations are prepared in various dosage forms, including *churna* (powders): *avaleha* (semi-solid preparations): *arishta* and *asava* (fermented liquid preparations): *ghrita* (medicated ghee): and *rasayana* formulations, which are primarily used for rejuvenation and enhancement of immunity (Dash & Junius, 2003). In recent decades, there has been a resurgence of interest in Ayurvedic medicines due to growing concerns about adverse effects, antimicrobial resistance, and high costs associated with synthetic pharmaceuticals. This renewed interest has led to increased commercialization and large-scale production of Ayurvedic formulations, raising concerns about quality, safety, and microbial contamination. Quality control is a critical aspect of pharmaceutical development, ensuring that medicinal products are safe, effective, and consistent. Unlike conventional pharmaceuticals, Ayurvedic formulations are often complex mixtures of multiple herbal ingredients, making standardization and quality assurance more challenging. Factors such as raw material quality, harvesting practices, processing methods, storage conditions, and packaging significantly influence the final product quality (Kunle et al., 2012). One of the major quality concerns in herbal and Ayurvedic medicines is microbiological contamination. Medicinal plants are natural products and are frequently exposed to environmental microorganisms during cultivation, harvesting, drying, and storage. Inadequate hygienic practices during manufacturing can further contribute to microbial load, potentially leading to product spoilage, reduced therapeutic efficacy, and health risks to consumers (WHO, 2007). Microbial contamination may include bacteria, fungi, yeast, and pathogenic organisms such as *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These contaminants can cause infections, especially in immunocompromised individuals, and may also produce toxic metabolites such as mycotoxins (Pandey et al., 2014).

Microbiological quality assessment is an essential component of herbal drug standardization. It involves evaluating the total microbial count, total yeast and mold count, and the absence or presence of specific pathogenic microorganisms. Regulatory bodies such as the WHO, Ayurvedic Pharmacopoeia of India (API): and pharmacopeias of different countries have established permissible limits for microbial contamination in herbal products (WHO, 2011). The WHO guidelines on quality control of herbal medicines specify acceptable microbial limits and recommend good agricultural and collection practices (GACP) and good

manufacturing practices (GMP) to minimize contamination. Despite these guidelines, many herbal formulations in the market still fail to meet microbial quality standards due to poor implementation and lack of routine monitoring (Chan, 2003).

Fermented Ayurvedic formulations such as *arishta* and *asava* pose unique microbiological challenges. While controlled fermentation contributes to therapeutic efficacy, uncontrolled microbial growth can compromise product safety. Therefore, microbiological evaluation of these formulations is particularly important to distinguish beneficial fermentation flora from harmful contaminants (Kulkarni et al., 2018).

1.2. Rasayana and Arishta Preparations in Ayurveda

In Ayurveda, Rasayana therapy is a specialized branch aimed at rejuvenation, longevity, immunity enhancement, and overall well-being. Rasayana formulations are often prescribed for chronic illnesses, convalescence, and geriatric care. These formulations may be consumed over prolonged periods, making their microbiological safety critically important (Shastri, 2005).

Arishta preparations are fermented herbal formulations containing self-generated alcohol, which acts as a preservative and enhances extraction of active constituents. Although alcohol content may inhibit certain microorganisms, improper fermentation or contamination during storage can still allow the growth of resistant microbial species (Kumar et al., 2016).

1.3. Overview of Amlokirasayana and Saribadyarista

Amlokirasayana is a classical Ayurvedic formulation traditionally used as a rejuvenative tonic. It is primarily indicated for improving digestion, enhancing immunity, promoting vitality, and supporting overall health. The formulation contains herbal ingredients rich in bioactive compounds such as polyphenols, flavonoids, and organic acids, which contribute to its therapeutic properties. Despite its widespread use, scientific data on the microbiological quality of Amlokirasayana is limited. The semi-solid or liquid nature of rasayana formulations may favor microbial growth if not properly processed or preserved. Therefore, systematic microbiological evaluation is essential to ensure product safety and compliance with pharmacopeial standards.

Saribadyarista is a well-known fermented Ayurvedic formulation commonly prescribed for skin disorders, blood purification, and inflammatory conditions. The formulation contains

multiple herbal ingredients and undergoes natural fermentation, resulting in alcohol formation that enhances shelf life and therapeutic potency. However, variations in fermentation conditions, raw material quality, and storage environments can significantly influence the microbial profile of Saribadyarista. Contamination with undesirable microorganisms may lead to product deterioration and potential health risks. Hence, microbiological quality assessment of Saribadyarista is essential to validate its safety and quality.

1.4. Rationale of the Study

With the growing global acceptance of Ayurvedic medicines, ensuring their microbiological safety has become a public health priority. Despite regulatory guidelines, there is a lack of comprehensive studies evaluating the microbiological quality of commonly used Ayurvedic formulations such as Amlokirasayana and Saribadyarista. This study aims to bridge this gap by conducting a systematic microbiological quality assessment of these formulations. The findings will contribute to standardization efforts, support regulatory compliance, and enhance consumer confidence in Ayurvedic medicines.

1.5. Global Scenario and Regulatory Perspectives on Herbal Medicines

The global herbal medicine market has expanded significantly over the past few decades, driven by increased consumer preference for natural therapies, preventive healthcare, and holistic treatment approaches. According to WHO estimates, nearly 80% of the world's population relies on traditional medicines for some aspect of primary healthcare (WHO, 2019). Ayurvedic formulations constitute a major segment of this global herbal market, with increasing exports to Europe, North America, and Southeast Asia. Despite their popularity, concerns regarding the quality, safety, and consistency of herbal medicines remain a major challenge. Regulatory frameworks for herbal medicines vary widely between countries. In India, Ayurvedic medicines are regulated under the Drugs and Cosmetics Act, 1940, and governed by the Ministry of AYUSH. Standards for raw materials, formulations, and quality parameters are documented in the Ayurvedic Pharmacopoeia of India (API). However, enforcement and routine quality monitoring remain inconsistent, particularly for microbiological parameters (Mukherjee, 2002).

Internationally, regulatory agencies such as the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) classify herbal products differently, often as dietary supplements rather than drugs. This classification may result in less stringent pre-

market quality evaluation, making post-market surveillance and microbiological assessment even more critical (Barnes, 2003).

1.6. Sources of Microbial Contamination in Ayurvedic Formulations

Microbial contamination in Ayurvedic formulations can occur at multiple stages of production. The primary sources include soil, water, air, raw plant materials, handling equipment, and personnel involved in manufacturing. Medicinal plants often harbor diverse microbial flora due to their direct exposure to environmental conditions during growth (De Smet, 2004). Improper drying of raw materials can lead to fungal growth, particularly molds such as *Aspergillus*, *Penicillium*, and *Fusarium* species. These fungi may produce mycotoxins that are stable even after processing and pose serious health risks upon consumption (Kumar & Singh, 2015). Additionally, the use of contaminated water during extraction or fermentation processes can introduce pathogenic bacteria into the formulations. Inadequate storage conditions, such as high humidity and temperature, further exacerbate microbial proliferation. Liquid and semi-solid Ayurvedic formulations, including rasayanas and arishtas, are especially susceptible due to higher water activity, which supports microbial growth if preservative mechanisms are insufficient (Prajapati et al., 2017).

1.7. Health Implications of Microbial Contamination

The presence of microbial contaminants in medicinal products can have serious implications for public health. Non-pathogenic microorganisms may cause product spoilage, unpleasant odor, and changes in appearance or taste, leading to reduced patient compliance. More importantly, pathogenic microorganisms can cause infections, particularly in vulnerable populations such as infants, elderly individuals, pregnant women, and immunocompromised patients (Okunlola et al., 2007). Certain bacteria, such as *Salmonella spp.* and *Escherichia coli*, are indicators of fecal contamination and poor hygienic practices. Their presence in medicinal formulations is unacceptable under pharmacopeial standards. Similarly, fungal contamination can result in exposure to mycotoxins, which have been associated with carcinogenic, hepatotoxic, nephrotoxic, and immunosuppressive effects (Bennett & Klich, 2003). Given that rasayana formulations are often consumed for prolonged durations as health supplements, even low-level microbial contamination may pose cumulative health risks. Therefore, ensuring microbiological safety is not only a regulatory requirement but also an ethical responsibility of manufacturers and healthcare providers.

1.8. Fermentation and Microbial Dynamics in Arishta Preparations

Fermentation is a distinctive feature of *arishta* formulations, where natural sugars from plant materials undergo microbial conversion to produce alcohol. This self-generated alcohol acts as a solvent for bioactive constituents and contributes to product preservation. However, fermentation is a biologically complex process that requires careful control to prevent contamination by undesirable microorganisms (Kulkarni & Datar, 2019). Beneficial yeast species are primarily responsible for fermentation, but contamination with pathogenic bacteria or spoilage fungi can occur if fermentation vessels, environmental conditions, or raw materials are not adequately controlled. The balance between beneficial and harmful microorganisms determines the final quality and safety of the product. Saribadyarista, being a polyherbal fermented preparation, presents additional complexity due to the interaction of multiple plant-derived compounds with microbial flora. Variability in fermentation practices across manufacturers can lead to significant differences in microbial profiles, highlighting the need for standardized microbiological evaluation.

1.9. Challenges in Standardization of Rasayana Formulations

Standardization of rasayana formulations such as Amlokirasayana is challenging due to variations in raw material composition, geographical sources, seasonal availability, and traditional preparation methods. Unlike synthetic drugs with single active ingredients, rasayanas contain multiple phytoconstituents that act synergistically (Mukherjee et al., 2010). Microbiological quality is an often-overlooked aspect of standardization. While physico-chemical parameters such as pH, viscosity, and organoleptic properties are commonly assessed, microbial load testing is not always routinely performed. This gap increases the risk of contaminated products reaching consumers. Furthermore, small-scale and traditional manufacturers may lack access to microbiological testing facilities, resulting in inconsistent quality control. Addressing these challenges requires increased awareness, capacity building, and integration of traditional knowledge with modern scientific methods.

1.10. Role of Good Manufacturing Practices (GMP)

Good Manufacturing Practices (GMP) play a crucial role in ensuring the quality and safety of pharmaceutical products, including Ayurvedic formulations. GMP guidelines emphasize cleanliness, controlled environments, proper documentation, trained personnel, and validated processes (WHO, 2014). Implementation of GMP in Ayurvedic pharmaceutical industries has improved product quality; however, compliance varies widely among manufacturers. Lack of infrastructure, insufficient training, and economic constraints are common barriers to

effective GMP implementation, particularly in small and medium-scale enterprises. Strict adherence to GMP can significantly reduce microbial contamination by minimizing exposure to contaminants during manufacturing, processing, and packaging. Therefore, microbiological quality assessment serves as an important tool to evaluate the effectiveness of GMP implementation in Ayurvedic industries.

1.11. Need for Scientific Validation of Ayurvedic Medicines

Scientific validation is essential for the global acceptance of Ayurvedic medicines. While traditional knowledge provides valuable insights into therapeutic efficacy, modern scientific methods are necessary to establish safety, quality, and reproducibility. Microbiological evaluation forms a critical component of this validation process (Patwardhan & Mashelkar, 2009). Studies focusing on microbiological quality assessment help identify potential risks and inform regulatory policies. They also contribute to the development of standardized protocols and quality benchmarks for Ayurvedic formulations. By generating empirical data, such studies bridge the gap between traditional medicine and modern pharmaceutical science.

1.12. Significance of the Present Study

The present study focuses on the microbiological quality assessment of two widely used Ayurvedic formulations: Amlokirasayana and Saribadyarista. These formulations are commonly prescribed for long-term use, making their safety profile particularly important. By evaluating total microbial count, fungal load, and presence of pathogenic microorganisms, this study provides valuable data on the microbiological safety of these formulations. The findings are expected to contribute to improved quality control practices, support regulatory compliance, and enhance consumer confidence in Ayurvedic medicines.

2.0. AIMS AND OBJECTIVES OF THE STUDY

2.1. General Objective

To assess the microbiological quality of fresh sugarcane juice sold by street vendors in Narayanganj, Bangladesh.

2.2. Specific Objectives

- To assess the total microbial load of Amlokirasayana and Saribadyarista.
- To evaluate the presence or absence of pathogenic microorganisms.
- To compare the microbial quality with established pharmacopeial standards.
- To ensure the safety and quality of the selected Ayurvedic formulations.

3.0 MATERIALS AND METHODS

3.1. Formulation of Amlokirasayana and Saribadyarista

The formulations (major ingredients) of Amlokirasayana and Saribadyarista according to the Bangladesh National Ayurvedic Formulary (BNAF). These are usually presented as the main herbal components used in preparing the formulations.

3.1.1. Amloki Rasayan Formula (BNFAM, 2011)

According to the Bangladesh National Ayurvedic Formulary, Amlokirasayana is mainly based on *Amloki (Emblica officinalis)* with digestive and carminative herbs used to improve gastrointestinal function. Typical ingredients include.

1. Amloki (*Phyllanthus emblica / Emblica officinalis*) – main ingredient
2. Pippali (*Piper longum*)
3. *Carum carvi* (Caraway)
4. *Cinnamomum zeylanicum* (Cinnamon)
5. *Syzygium aromaticum* (Clove)
6. *Trachyspermum ammi* (Ajwain)
7. *Zingiber officinale* (Ginger)
8. Sugar or jaggery base (vehicle)
9. Water or herbal decoction base

3.1.2. Composition (per 5 ml extract) of of Amlokirasayana

- *Carum carvi* – 2.64 g
- *Cinnamomum zeylanicum* – 13.24 mg
- *Syzygium aromaticum* – 13.24 mg
- *Trachyspermum ammi* – 13.24 mg
- *Zingiber officinale* – 26.48 mg
- Other ingredients q.s.

3.1.3. Therapeutic use of Amlokirasayana

- Hyperacidity
- Dyspepsia
- Indigestion
- Flatulence
- Gastric and duodenal ulcer

3.2. Saribadyarista Formula (BNFAM, 2011)

Saribadyarista is a fermented Ayurvedic liquid preparation (Arishta) mainly used for blood purification and skin diseases. Typical ingredients according to BNAF-based formulations include.

1. Sariba (Hemidesmus indicus)
2. Ichnocarpus frutescens
3. Curcuma longa (Turmeric)
4. Azadirachta indica (Neem)
5. Tinospora cordifolia (Guduchi)
6. Swertia chirata (Chirata)
7. Andrographis paniculata (Kalmegh)
8. Aphanamixis polystachya
9. Phyllanthus emblica (Amloki)
10. Pterocarpus santalinus (Red sandalwood)
11. Jaggery or sugar (fermentation medium)
12. Water for decoction

3.2.1. Composition (per 5 ml) Saribadyarista

- *Hemidesmus indicus* – 78.13 mg
- *Ichnocarpus frutescens* – 78.13 mg
- *Curcuma longa* – 78.13 mg
- *Azadirachta indica* – 78.13 mg
- *Tinospora cordifolia* – 78.13 mg
- *Swertia chirata* – 78.13 mg
- *Andrographis paniculata* – 78.13 mg
- *Aphanamixis polystachya* – 78.13 mg
- *Phyllanthus emblica* – 78.13 mg
- *Pterocarpus santalinus* – 15.63 mg

3.2.2. Therapeutic use of Saribadyarista

- Skin diseases (scabies, eczema, dermatitis)
- Blood purification

- Boils and pimples
- Liver detoxification and inflammatory disorders

3.3. Study Area

Ayurvedic formulation Amlokirasayana and Saribadyarista were examined for microbiological quality analysis.

3.4. Sample Size

10 Ayurvedic polyherbal formulation (10 Amlokirasayana and 10 Saribadyarista).

3.5. Sample Collection Method

Twenty samples of Ayurvedic formulation Amlokirasayana and Saribadyarista were examined for microbiological quality analysis collected from local market of Dhaka city, Bangladesh and transported to the laboratory.

3.6. Sample Processing

The samples were in liquid form, so further processing was not required. One ml of liquid sample was taken and transferred into sterilized cotton plugged test tubes containing 9 ml of 0.1% peptone water. Then they were mixed thoroughly by shaking 20 times. This time the solution was allowed to stand for 5-10 minutes. Thus, initial dilution of homogenate was prepared and from this homogenization further serial dilution were prepared.

3.7. Microbiological Study

The microbiological quality of the formulations was evaluated by determining the Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC) using the standard pour plate method, as described by the American Public Health Association (APHA, 1992) and the World Health Organization (WHO, 2011). Serial tenfold dilutions (10^{-1}–10^{-6}) of each sample were prepared using sterile diluent in accordance with APHA guidelines (APHA, 1992). Aliquots of the diluted samples were inoculated onto Plate Count Agar (PCA) for the enumeration of aerobic bacteria and Potato Dextrose Agar (PDA) for the enumeration of yeasts and molds. Duplicate plates were prepared for each dilution to ensure the accuracy and reproducibility of the results (APHA, 1992).

The bacterial culture plates were incubated at 36 ± 2°C for 44 ± 4 h, whereas the yeast and mold plates were incubated at 22 ± 2°C for 68 ± 4 h. After incubation, colonies were counted manually, and only countable plates containing 30–300 colonies for bacteria and 10–150

colonies for yeasts and molds were considered for enumeration (United States Pharmacopeia (USP, 2023). The microbial load was expressed as colony-forming units per millilitre (CFU/mL) by multiplying the average colony count by the corresponding dilution factor.

The microbial count was calculated using the following equation:

$$CFU/mL = \frac{\text{Number of colony x Dilution Factor}}{\text{Volume of Inoculum (ml)}}$$

4.0. RESULTS AND DISCUSTIONS

4.1. Result: Microbial Quality of Amlokirasayana and Saribadyarista

Table 4.1: Microbiological Quality Assessment of Amlokirasayana (AMR) Samples

The microbiological quality assessment of the AMR samples demonstrated that the Total Aerobic Microbial Count (TAMC) ranged from 1×10^1 to 1×10^4 CFU/mL, while the Total Yeast and Mold Count (TYMC) ranged from 1×10^1 to 1×10^3 CFU/mL. All samples complied with the recommended microbiological limits of $\leq1\times10^5$ CFU/mL for TAMC and $\leq1\times10^3$ CFU/mL for TYMC. Although sample AMR-3 reached the maximum permissible TYMC limit (1×10^3 CFU/mL): it remained within the acceptable specification. Overall, the results indicate that all AMR samples meet the required microbiological quality standards and are considered microbiologically acceptable for use.

Samples	Total Aerobic Microbial Count (TAMC) (CFU/mL)	Acceptable Limit (CFU/mL)	Total Yeast and Mold Count (TYMC) (CFU/mL)	Acceptable Limit (CFU/mL)
AMR-1	1×10^3	$\leq 1 \times 10^5$	1×10^2	$\leq 1 \times 10^3$
AMR-2	1×10^3		1×10^2	
AMR-3	1×10^2		1×10^3	
AMR-4	1×10^4		1×10^1	
AMR-5	1×10^2		1×10^1	
AMR-6	1×10^4		1×10^1	
AMR-7	1×10^1		1×10^2	
AMR-8	1×10^3		1×10^1	
AMR-9	1×10^3		1×10^2	
AMR-10	1×10^4		1×10^1	

Here AMR means Amlokirasayana

Table 4.2: Microbiological Quality Assessment of Saribadyarista (SBR) Samples Based on Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC).

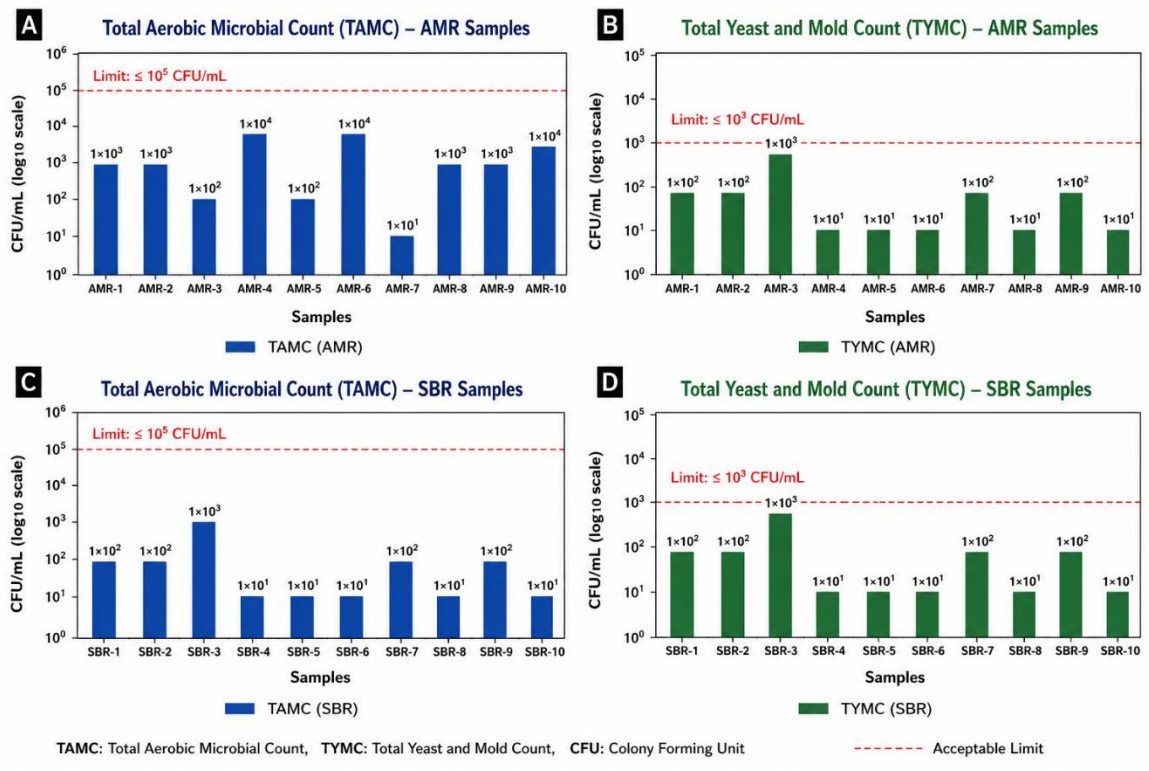
Sample	Total Aerobic Microbial Count (TAMC) (CFU/mL)	Acceptable Limit (CFU/mL)	Total Yeast and Mold Count (TYMC) (CFU/mL)	Acceptable Limit (CFU/mL)
SBR-1	1×10^2	$\leq 1 \times 10^5$	1×10^2	$\leq 1 \times 10^3$
SBR-2	1×10^2		1×10^2	
SBR-3	1×10^3		1×10^3	
SBR-4	1×10^1		1×10^1	
SBR-5	1×10^1		1×10^1	
SBR-6	1×10^1		1×10^1	
SBR-7	1×10^2		1×10^2	
SBR-8	1×10^1		1×10^1	
SBR-9	1×10^2		1×10^2	
SBR-10	1×10^1		1×10^1	

SBR means Saribadyarista

The microbiological evaluation of the SBR samples revealed that the Total Aerobic Microbial Count (TAMC) ranged from 1×10^1 to 1×10^3 CFU/mL, while the Total Yeast and Mold Count (TYMC) ranged from 1×10^1 to 1×10^3 CFU/mL. All samples complied with the pharmacopeial acceptance criteria of $\leq 1 \times 10^5$ CFU/mL for TAMC and $\leq 1 \times 10^3$ CFU/mL for TYMC. Although SBR-3 exhibited the highest TYMC (1×10^3 CFU/mL): it remained within the permissible limit. Overall, the findings indicate that all SBR samples satisfied the microbiological quality requirements and were considered acceptable with respect to bacterial, yeast, and mold contamination.

4.2. DISCUSSION

The microbiological quality of herbal medicinal products is an important aspect of their safety, stability, and therapeutic effectiveness. Ayurvedic formulations, which are derived primarily from plant materials and natural ingredients, are particularly susceptible to microbial contamination during various stages such as harvesting of raw materials, processing, manufacturing, storage, and distribution. Therefore, evaluating the microbial load of these products is essential to ensure that they meet established quality standards and are safe for human consumption. The present study assessed the microbiological quality of two widely used Ayurvedic formulations, Amlokirasayana and Saribadyarista, by determining the total viable bacterial count and the total yeast and mold count in multiple samples.



Graph 4.1. Microbiological Quality Assessment of Amlokirasayana and Saribadyarista Based on Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC).

The total aerobic microbial count (TAMC) and total yeast and mold count (TYMC) are commonly used indicators to evaluate microbial contamination in pharmaceutical and herbal products. According to established pharmacopeial guidelines for herbal medicinal preparations, the acceptable limits are $\leq 1 \times 10^5$ CFU/mL for total aerobic microbial count and $\leq 1 \times 10^3$ CFU/mL for total yeast and mold count. These limits are considered safe thresholds beyond which microbial contamination may compromise the quality and safety of the product. In this study, the microbial counts obtained from all tested samples of Amlokirasayana and Saribadyarista were compared with these standard limits to determine their microbiological acceptability.

The results for Amlokirasayana indicated that the total viable bacterial count ranged from 1×10^1 to 1×10^4 CFU/mL across the ten analyzed samples. Among these samples, AMR-4, AMR-6, and AMR-10 showed relatively higher bacterial counts of 1×10^4 CFU/mL compared to the other samples, while AMR-7 exhibited the lowest count of 1×10^1 CFU/mL. Despite these variations, all bacterial counts remained well below the permissible limit of 1×10^5 CFU/mL. This suggests that the bacterial contamination present in the samples was

minimal and within acceptable microbiological standards. Variations in bacterial counts among different samples may be attributed to differences in raw material quality, environmental conditions during processing, or handling practices during manufacturing and storage.

Similarly, the total yeast and mold counts observed in Amlokirasayana samples ranged from 1×10^1 to 1×10^3 CFU/mL. Some samples such as AMR-3 and AMR-7 showed relatively higher yeast and mold counts compared to others. However, the maximum observed value of 1×10^3 CFU/mL still falls within the acceptable limit for fungal contamination in herbal medicinal products. Yeasts and molds are common contaminants in plant-based products due to their ability to grow in environments with moderate moisture and organic nutrients. Their presence in low numbers is not uncommon in herbal preparations, but maintaining counts within acceptable limits is crucial to prevent spoilage and potential health risks.

The microbiological evaluation of Saribadyarista samples also demonstrated satisfactory results. The total viable bacterial counts ranged from 1×10^1 to 1×10^3 CFU/mL, which are significantly lower than the maximum permissible limit of 1×10^5 CFU/mL. Samples such as SBR-3 showed comparatively higher bacterial counts (1×10^3 CFU/mL): while several samples including SBR-4, SBR-5, SBR-6, SBR-8, and SBR-10 exhibited very low bacterial counts of 1×10^1 CFU/mL. These results indicate that the microbial load in Saribadyarista samples is minimal and reflects good manufacturing and hygienic practices during production.

The total yeast and mold counts in Saribadyarista samples ranged from 1×10^1 to 1×10^3 CFU/mL. Similar to the bacterial results, most samples showed low fungal counts, indicating effective control of fungal contamination. The presence of yeast in Saribadyarista may partly be associated with the natural fermentation process involved in the preparation of many Ayurvedic arishta formulations. Fermentation is a characteristic step in the preparation of arishta and asava products, where natural microbial activity contributes to the development of certain therapeutic properties and stability of the formulation. However, excessive microbial growth can compromise product quality, making routine monitoring essential.

The comparatively low microbial counts observed in both formulations may also be attributed to the inherent antimicrobial properties of certain herbal ingredients present in these preparations. Many medicinal plants used in Ayurvedic formulations contain bioactive

compounds such as phenolics, tannins, flavonoids, and essential oils that exhibit antimicrobial activity. These compounds can inhibit the growth of bacteria and fungi, thereby contributing to the overall microbiological stability of the product. Additionally, certain preparation methods such as boiling, fermentation, and the addition of natural preservatives may further reduce microbial contamination.

Another factor that may influence microbial quality is the level of moisture and pH of the formulations. Products with lower water activity and acidic pH conditions generally inhibit the growth of many pathogenic microorganisms. Arishta preparations like Saribadyarista often possess slightly acidic conditions due to fermentation, which may contribute to limiting microbial proliferation. Similarly, the processing techniques used in the preparation of Amlokirasayana, including heating and concentration of herbal extracts, may help reduce microbial contamination.

Although the microbial counts obtained in this study were within acceptable limits, the observed variations among samples highlight the importance of maintaining strict quality control measures throughout the production process. Good Manufacturing Practices (GMP): proper sanitation of equipment, hygienic handling of raw materials, and appropriate storage conditions are essential to minimize microbial contamination. Regular microbiological testing should be incorporated into routine quality assurance procedures to ensure that herbal medicinal products consistently meet established safety standards.

Furthermore, monitoring microbial quality is particularly important because certain microorganisms may produce toxins or cause spoilage if present in high numbers. Although the current study focused on total microbial counts, future studies could include the detection of specific pathogenic microorganisms such as *Escherichia coli, Salmonella species, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The absence of such pathogens is a critical requirement for ensuring the safety of pharmaceutical and herbal products.

Overall, the results of this investigation demonstrate that both Amlokirasayana and Saribadyarista formulations possess satisfactory microbiological quality. All tested samples exhibited microbial counts well within the acceptable pharmacopeial limits for herbal medicinal products. These findings suggest that the manufacturing processes, handling practices, and storage conditions associated with these formulations are adequate to maintain their microbiological safety.

The microbiological assessment conducted in this study confirms that the tested samples of Amlokirasayana and Saribadyarista are safe in terms of microbial contamination and comply with the recommended standards for herbal medicinal preparations. Continuous monitoring and adherence to strict quality control practices remain essential to ensure the safety, efficacy, and reliability of Ayurvedic formulations. Such evaluations not only support consumer safety but also strengthen confidence in traditional herbal medicine systems by demonstrating their compliance with modern scientific quality standards.

5.0. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

The present study was conducted to evaluate the microbiological quality of two widely used Ayurvedic formulations, Amlokirasayana and Saribadyarista, by determining their total viable bacterial count and total yeast and mold count. Microbiological assessment is an essential component of quality control in herbal medicinal products because these formulations are derived from natural sources that may be susceptible to microbial contamination during harvesting, processing, manufacturing, storage, and distribution. Ensuring that microbial levels remain within acceptable limits is therefore crucial for maintaining product safety, stability, and therapeutic efficacy. The microbiological evaluation carried out in this study demonstrates that the analyzed samples of Amlokirasayana and Saribadyarista meet the acceptable microbial limits for herbal medicinal products. These findings support the microbiological safety of the formulations and highlight the importance of regular quality assessment to ensure consumer safety and maintain confidence in traditional Ayurvedic medicine.

5.2. Recommendations

It is recommended that manufacturers continue to adhere to Good Manufacturing Practices (GMP): maintain stringent hygiene during production, and implement regular microbiological quality control testing to ensure product safety and consistency. Further studies involving pathogenic microbial screening, stability evaluation, and multi-batch quality assessment are also recommended to strengthen the microbiological safety and overall quality assurance of these Ayurvedic formulations.

6. Conflicts of interests

The authors do not disclose any conflicting interests.

7. ACKNOWLEDGMENT

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