https://doi.org/10.33451/florafauna.v32i1pp121-126
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 121-126 ISSN 0971 - 6920 (Print)

Microbial and heavy metal profiling of marketed *Jeerakarishta* : ensuring safety and standardization of a traditional Ayurvedic formulation

Sumeet Dwivedi and *Sweta S Koka

Acropolis Institute of Pharmaceutical Education and Research,
INDORE-453771 (MP) INDIA
*Correspondence Author
E-mail : sweta.koka@gmail.com

Received **:** 11.03.2026; ***Accepted*** : 08.05.2026

How to cite : Dwivedi S, Koka SS. Microbial and heavy metal profiling of marketed *Jeerakarishta* : ensuring safety and standardization of a traditional Ayurvedic formulation. *Flora and Fauna* 2026. 32(1) : 121-126.

ABSTRACT

Jeerakarishta, a classical Ayurvedic fermented formulation (Arishta), is widely prescribed for digestive disorders and postnatal care. This study conducted a comprehensive quality evaluation of two commercially available *Jeerakarishta* formulations following strict guidelines from the *Ayurvedic Pharmacopoeia of India* (AFI), World Health Organization (WHO), and Central Council for Research in Ayurvedic Sciences (CCRAS). The evaluation encompassed organoleptic, physicochemical, chromatographic, microbial, and toxicological parameters. Results indicated compliance with standard limits for most parameters, though alcohol content (27% v/v) exceeded the AFI limit (d"12% v/v). Heavy metals (As, Cd, Hg, Pb) were within permissible limits (As: 0.13-0.22 ppm, Cd: 0.0007-0.02 ppm, Hg: 0.003-0.08 ppm, Pb: 0.002-0.11 ppm). Microbial counts were within acceptable ranges (TBC <10u CFU/mL, TFC <10^3 CFU/mL). Both formulations demonstrated acceptable quality and safety profiles, though variations in physicochemical parameters suggest differences in manufacturing processes. This study underscores the importance of systematic quality control in ensuring the safety and efficacy of Ayurvedic formulations.

Figure : 00 References : 20 Tables : 04

KEY WORDS : AFI guidelines, Ayurvedic formulation, CCRAS standards, Heavy metal analysis, *Jeerakarishta*, Microbial contamination, Quality control, WHO protocols.

Introduction

Ayurveda, the ancient Indian system of medicine, has experienced a global resurgence due to its holistic approach and perceived safety. Among its diverse pharmaceutical preparations, *Asava* and *Arishta* represent unique fermented dosage forms prepared through *sandhanakalpana*, a process involving self-generated alcohol fermentation without external alcohol addition. These formulations are valued for their extended shelf life, enhanced therapeutic efficacy, and improved bioavailability due to the presence of self-generated ethanol, which acts as both a preservative and a solvent for active constituents[12,13].

Jeerakarishta, a classical Ayurvedic *Arishta*, is primarily indicated for digestive disorders (*ajirna*, *grahani*), postnatal weakness (*sutika kala*), poor appetite (*aruchi*), and gastrointestinal disturbances. Its chief ingredient, *Cuminum cyminum* (Jeeraka), is renowned for its *deepana* (appetizer) and *pachana* (digestive) properties. The formulation typically contains other medicinal herbs including Patha (*Cissampelos pareira*), Ela (*Elettaria cardamomum*), Mustaka (*Cyperus rotundus*), Shunthi (*Zingiber officinale*) and Dhataki flowers (*Woodfordia fruticosa*) which act as natural fermenting agents[15]. According to the *Ayurvedic Pharmacopoeia of India* (API), *Jeerakarishta* should

ACKNOWLEDGEMENTS : The authors gratefully acknowledge the Acropolis Institute of Pharmaceutical Education and Research, Indore, for providing laboratory facilities and technical support. Special thanks to the Food and Testing Laboratory, Acropolis, for assistance with microbial and heavy metal analyses. We also thank the Department of AYUSH, Government of Madhya Pradesh, for guidance on regulatory requirements.

TABLE-1 : Organoleptic parameters of *Jeerakarishta* samples

Parameter	Sample A	Sample B	Classical Description
Color	Brown to dark brown	Brown to reddish brown	Brown, dark brown
Odor	Pleasant, alcoholic, distinct cumin aroma	Pleasant, alcoholic, mild cumin aroma	Alcoholic, aromatic
Taste	Sweet with astringent-spicy aftertaste	Sweet with less astringency	Madhura, Kashaya, Katu anurasa

contain 5-10% self-generated alcohol and comply with specified physicochemical and microbial limits[15].

The World Health Organization (WHO) emphasizes that herbal medicines must be evaluated for identity, purity, strength, and safety through comprehensive quality control measures[16,19]. Similarly, the Central Council for Research in Ayurvedic Sciences (CCRAS) and the *Ayurvedic Formulary of India* (AFI) have established detailed guidelines for the standardization of Ayurvedic drugs, covering botanical, physicochemical, toxicological, and chromatographic parameters[1,3].

Materials and Methods

2.1. Sample Collection and Coding

Two commercially available brands of *Jeerakarishta* were procured from licensed pharmacies in Indore, Madhya Pradesh. Samples were stored at 25±2°C in their original amber-colored glass bottles until analysis. All analyses were initiated within one week of procurement.

2.2. Organoleptic Evaluation

Organoleptic assessment was performed according to WHO Guidelines for the Assessment of Herbal Medicines (1996) and CCRAS Guidelines for Drug Development of Ayurvedic Formulations[3]. In this study color, odor and taste assessment were done as per guidelines.

2.3. Physicochemical Analysis: All physicochemical analyses were performed in triplicate following strict standard operating procedures. Instrument calibration was verified before each analysis session.

2.3.1. Determination of Total Solid Content: Total solid content was determined using two methods. For sugar-containing formulations, 50 mL of sample was evaporated, extracted with dehydrated ethanol, re-evaporated with diatomite, dried at 105°C, and weighed after cooling. For sugar-free preparations, 50 mL was directly evaporated to dryness, dried at 105°C for 3 hours, cooled, and weighed to calculate total solids[14].

2.3.2. Determination of Specific Gravity[8] : Specific gravity was measured using a calibrated pycnometer at 25°C. The weights of the pycnometer filled with the sample (W) and with distilled water (W,) were recorded.

2.3.3. Determination of Viscosity: Viscosity was measured using an Ostwald viscometer at 25°C by recording the flow time of the sample between two marks and comparing it with distilled water under identical conditions. The densities of the sample and water were determined using a pycnometer, and viscosity was calculated relative to water.

2.3.4. Determination of Alcohol Content[14]: Alcohol content was estimated using the Distillation Method (Method IIIA) as per CCRAS guidelines. A 25 mL sample was diluted with 150 mL of distilled water and distilled, collecting at least 90 mL of distillate in a 100 mL volumetric flask. The distillate was adjusted to 25°C, diluted to volume, and its specific gravity was measured. The alcohol percentage was determined by referring to standard alcoholometric tables.

2.3.5. Determination of pH[8]: pH was determined by pH meter.

2.3.6. Determination of Reducing Sugar Content[18]: Reducing sugar content was estimated by the Lane–Eynon titrimetric method. Fehling's solution was first standardized using 0.5% standard dextrose with methylene blue indicator to a brick-red endpoint. The appropriately diluted sample was then titrated similarly, and reducing sugar content was calculated based on the recorded titration volumes.

TABLE-2 : Physicochemical parameters of *Jeerakarishta* samples

Parameter	Standard Requirement	Sample A	Sample B
Total Solid Content (% w/v)	≥ 20	29.0±0.5	22.7±0.4
Specific Gravity	1.080-1.120	1.150±0.005	1.084±0.003
Viscosity (cP)	1.2-1.5	1.5±0.1	1.3±0.1
Alcohol Content (% v/v)	NMT 12	27.0±0.5	27.0±0.5
pH	4.0-4.5	3.9±0.1	4.2±0.1
Reducing Sugar (% w/v)	NLT 5	0.6242±0.02	0.6645±0.02
Refractive Index	1.3320-1.3420	1.412±0.0005	1.3652±0.0005
Acid Value	NMT 7	5.2±0.2	6.3±0.2
TLC (Rf of thymol)	~0.59	0.59±0.02	0.59±0.02

Values represent mean ± standard deviation (n=3)

2.3.7. Determination of Refractive Index[14]: Refractive index was measured using an Abbe refractometer calibrated with distilled water (1.3325 at 25°C). After cleaning the prisms, 2–3 drops of sample were placed on the prism, equilibrated at 25°C, and the boundary line was adjusted for a clear reading. The value was noted directly from the scale, and prisms were cleaned after measurement.

2.3.8. Determination of Acid Value[6]: The acid value was determined titrimetrically. A 10 mL sample was dissolved in 50 mL of an ether-alcohol mixture (1:1) and titrated with 0.1 N NaOH using phenolphthalein as the indicator.

2.3.9. Chromatographic Analysis (Thin Layer Chromatography for Thymol)[20]: TLC was performed as per AFI guidelines. Silica gel G plates were activated at 105°C for 30 minutes. Sample and standard thymol solutions were spotted and developed in a mobile phase of toluene:ethyl acetate (9:1). The plates were visualized under UV light (254 nm) and after iodine staining, and Rf values were calculated.

2.4. Toxicological Analysis

2.4.1. Microbial Quality Analysis: All microbial analyses were conducted in a certified microbiology laboratory under laminar airflow (Class II). Media preparation, sterilization, and incubation followed Ministry of AYUSH Guidelines for Microbial Limit Testing (2016) and WHO Guidelines on Quality Control of Herbal Medicines (2007).

A. Total Bacterial Count (TBC) – Aerobic Mesophilic Bacteria: For Total Bacterial Count, 10 mL of sample was diluted in 90 mL Buffered Peptone Water to obtain a 10{ ¹ dilution, followed by serial dilutions as required. Using the pour plate method, 1 mL of selected dilutions was mixed with molten SCDA and incubated at 30–35°C for 5 days. Plates with 30–300 colonies were counted, and TBC was calculated using the dilution factor, with appropriate positive and negative controls maintained.

B. Total Fungal Count (TFC) – Yeasts and Molds: For Total Fungal Count, serially diluted samples were plated in duplicate using the pour plate method with molten Sabouraud Dextrose Agar (d”48°C). After solidification, plates were incubated at 20–25°C for 5 days. Yeast and mold colonies were counted separately to determine the total fungal load.

C. Detection of *Enterobacteriaceae*: For detection

TABLE-3 : Microbial quality parameters of *Jeerakarishta* samples

Parameter	Permissible Limit (AYUSH)	Sample A	Sample B
Total Bacterial Count	≤10 CFU/mL	<100 CFU/mL	<100 CFU/mL
Total Fungal Count	≤10^3 CFU/mL	<10 CFU/mL	<10 CFU/mL
Enterobacteriaceae	Absent in 1 mL	Absent	Absent

of Enterobacteriaceae, 10 mL of sample was pre-enriched in 90 mL Buffered Peptone Water and incubated at 37°C for 18–24 hours. The enriched broth was streaked onto VRBGA plates and incubated at 37°C for 24±2 hours. Typical pink to red colonies with bile precipitation were observed, followed by confirmatory tests including Gram staining, oxidase test, and IMViC reactions for identification.

2.4.2. Heavy Metal Analysis [ICP-OES Instrumentation and Analysis]

For heavy metal analysis, 5 mL of sample was digested with concentrated HNO following a pre-digestion step, then heated gradually (60–120°C) until a clear solution was obtained. If required, H_2O, was added for complete oxidation. The digest was reduced to 2–3 mL, cooled, diluted to 50 mL with ultrapure water, and filtered through a 0.45 µm membrane. Samples were stored at 4°C and analyzed within 24 hours using ICP techniques.

Results and Discussion

3.1. Organoleptic Evaluation

Both *Jeerakarishta* formulations showed characteristic organoleptic properties (Table-1) consistent with classical descriptions. The brown to dark brown color reflects proper fermentation and sugar caramelization, while the pleasant alcoholic aroma with cumin notes confirms self-generated ethanol and volatile oils. The sweet taste with slight astringent and spicy aftertaste aligns with the traditional rasa profile.

3.2. Physicochemical Parameters

Physicochemical parameters (Table-2) largely complied with pharmacopoeial standards; however, alcohol content (27% v/v) exceeded the AFI limit (d”12%). This may result from prolonged fermentation or raw material variations. While higher alcohol could improve preservation, it might influence palatability and dosage considerations.

The total solid content was within acceptable limits, indicating efficient extraction and concentration. Sample A showed slightly higher specific gravity (1.150), likely due to increased soluble solids. Low reducing sugar content suggests effective fermentation of sugars to alcohol, while the acidic pH (3.9–4.2) and moderate acid values (5.2–6.3) support stability and preservation. TLC analysis confirmed the presence of thymol (Rf 0.59), verifying the authenticity of cumin in both formulations.

3.3. Microbial Quality

Both formulations demonstrated excellent microbial quality (Table-3), with counts well below permissible limits. The absence of *Enterobacteriaceae* indicates proper hygienic practices during manufacturing and packaging. The low microbial load can be attributed to the self-generated alcohol (27% v/v) which acts as a natural preservative, and the acidic pH (3.9-4.2) which inhibits bacterial growth.

3.4. Heavy Metal Analysis

Heavy metal concentrations were within permissible limits for all elements tested (Table-4). The detected levels are likely from environmental contamination of herbal raw materials rather than intentional addition. The relatively higher arsenic content in Sample B (0.22 ppm) compared to Sample A (0.13 ppm) may reflect geographical variations in raw material sourcing. All values were significantly below the maximum permissible limits, ensuring product safety.

The ICP-OES calibration curves showed excellent linearity with correlation coefficients (r^2) e”0.999 for all elements (Figure 1). Method validation parameters met acceptance criteria: precision (RSD <10%), accuracy (recovery 92-108%), and detection limits below 1% of permissible limits.

Discussion

The comprehensive quality evaluation of Jeerakarishta formulations highlights key issues in the standardization of Ayurvedic fermented preparations. Both samples showed elevated alcohol content (27% v/v), exceeding the AFI limit of d”12% v/v. In traditional

TABLE-4 : Heavy metal content in *Jeerakarishta* samples (ppm)

Metal	Permissible Limit	Sample A	Sample B
Arsenic (As)	0.3	0.13±0.01	0.22±0.02
Cadmium (Cd)	0.03	0.020±0.002	0.0007±0.0001
Mercury (Hg)	0.1	0.080±0.005	0.003±0.001
Lead (Pb)	1.0	0.110±0.010	0.002±0.001

Values represent mean ± standard deviation (n=3)

practice, alcohol is self-generated through natural fermentation of sugars from jaggery or honey, and its final concentration depends on factors such as sugar content, fermentation duration, temperature, and microbial flora. Although classical texts acknowledge seasonal and geographical variations, the higher alcohol levels observed may influence dosage considerations and patient acceptability, especially among individuals with alcohol restrictions. Inter-brand differences in total solids, specific gravity, and refractive index further reflect variability in raw material quality, fermentation conditions, processing methods, and batch consistency, underscoring the need for stricter in-process controls and standardized manufacturing protocols.

The microbial and heavy metal analyses confirm the safety of both formulations. The self-generated alcohol and acidic pH create an unfavorable environment for microbial growth, contributing to excellent microbiological quality. Heavy metal levels, though detectable, remained well within regulatory safety limits; however, routine monitoring remains essential due to the potential accumulation of metals in herbal raw materials from contaminated soil and water. The study employed validated physicochemical methods alongside AYUSH- and WHO-aligned microbial testing and sensitive ICP-OES analysis for heavy metals, ensuring reliable, accurate, and internationally acceptable quality assessment.

Conclusion

The study confirms that both *Jeerakarishta* formulations largely comply with AFI, WHO, and CCRAS quality standards, exhibiting characteristic organoleptic properties and acceptable physicochemical parameters. Microbial load and heavy metal levels were within safe limits, confirming product safety. However, alcohol content exceeded prescribed limits and inter-brand variations were observed, indicating the need for stricter fermentation control and standardization. Overall, the formulations are safe and of good quality, though improved process monitoring and advanced phytochemical profiling are recommended for enhanced consistency and therapeutic reliability

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Ethical Statement

This study involved analysis of commercial products only and did not involve human or animal subjects. All analyses were conducted following standard laboratory safety protocols.

References

1. Agarwal SS. *Herbal Drug Technology*. Universities Press, Hyderabad. 2007.
2. AOAC International. *AOAC Official Method 2015.01: Heavy Metal Analysis Using ICP-OES*. AOAC International. 2015.
3. Central Council for Research in Ayurvedic Sciences. *General Guidelines for Drug Development of Ayurvedic Formulations.* 2010; **1** : Ministry of AYUSH, Government of India.
4. Central Council for Research in Ayurvedic Sciences. *Validation Guidelines for Analytical Methods*. Ministry of

AYUSH, Government of India. 2020.

5. Chakravarthy BK. Standardization of Herbal Products. *Indian Journal of Natural Products*. 1993; **9**(1) : 23-26.
6. Chemil B. *Belter's Textbook of Pharmaceutical Chemistry*, 15th Edition. Reno Books, USA. 1995.
7. Choudhary RD. *Herbal Drug Technology*. Eastern Publishers, New Delhi. 1996.
8. Indian Pharmacopoeia Commission. *Indian Pharmacopoeia 2018*. Ministry of Health and Family Welfare, Government of India. 2018.
9. Ministry of AYUSH. *Guidelines for Microbial Limit Test as per Ayurvedic Pharmacopoeia of India*. Government of India. 2016.
10. Ministry of AYUSH. *Guidelines for Safety Evaluation of ASU Drugs*. Government of India. 2018.
11. Mukherjee PK. *Quality Control of Herbal Drugs*. Business Horizon, New Delhi. 1999.
12. Pandey MN, Agarwal P. Standardization of Ayurvedic Drugs. *Sachitra Ayurveda*. 2006; **1**(1) : 45-52.
13. Sane RT. Standardization, Quality Control and GMP for Herbal Drugs. *Indian Drugs*. 2002; **39**(4) : 184-190.
14. Subramanyam CVS. *Essentials of Physical Pharmacy*. Vallabh Prakashan, New Delhi. 1997.
15. The Ayurvedic Formulary of India. Government of India. 2003 **1**(2 Rev. Ed.).
16. The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of AYUSH. 2008; **1**(3) : 29.
17. The Ayurvedic Pharmacopoeia of India, General Guidelines for Evaluation. Government of India.
18. The Ayurvedic Procedures of India. Government of India. 2005; **1**(6 Edi.).
19. World Health Organization. *WHO Guidelines on Quality Control of Herbal Medicines*. Geneva: WHO Press. 2007.
20. Zhang L, Liao C. *HPTLC and TLC Methods in Herbal Analysis*. Springer-Verlag, Berlin. 2005.