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

International Journal of Ayurveda and Pharma Research

Research Article

COMPARISON OF COLONY FORMING UNITS (CFU) BETWEEN PREPARED AND MARKET SAMPLES OF SELECTED *ARISTAS* IN THE KERALA MARKET

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Article info

Article History:
Received: 28-12-2024
Accepted: 15-01-2025
Published: 07-02-2025

KEYWORDS:
Abhayarista, Balarista, Colony Forming Units, *Jeerakarista*.

ABSTRACT

The study compares the Colony Forming Units (CFU) of prepared and market samples of three *Aristas*: *Abhayarista, Balarista,* and *Jeerakarista*, which are fermented Ayurvedic medicines. *Aristas* naturally generate alcohol through fermentation facilitated by yeast found in *Woodfordia fruticosa* Kurz, with Jaggery serving as the sugar source. The fermentation process spans 30-45 days, during which complete sugar utilization ensures optimal results. Incomplete fermentation, however, can cause post-fermentation issues and acidity development. The microbial fermentation process is characterized by distinct growth phases: a lag phase for acclimatization, a log phase for exponential growth, a deceleration phase due to reduced energy reserves, and a stationary phase where microbial activity stagnates but viability is maintained. The presence of residual sugar can trigger renewed fermentation cycles, leading to post-fermentation challenges. For this analysis, three samples of prepared *Aristas* and five corresponding market samples from the Kerala market were tested. CFU was assessed using the Mueller-Hinton agar method, and statistical evaluation employed a one-sample t-test. The results revealed a significant difference in CFU levels in two of the market samples ($p < 0.01$), while the remaining samples showed no significant variation ($p>0.05$). This study highlights the variability in CFU between prepared and market samples, emphasizing the importance of consistent fermentation processes in ensuring the quality of Ayurvedic fermented medicines.

INTRODUCTION

Aristas are fermented, liquid, self-preserved alcoholic formulation. [1] The sweet taste and pleasant aroma makes it a widely accepted form of medication [2]. Fermentation here is mediated by the wild species of yeast present in the flowers of *Woodfordia fruticosa* Kurz, which acts as the fermentation initiator.[3] Quality control of Ayurvedic formulations is of great significance in determining its therapeutic efficacy and shelf life[4]. Colony forming units generally helps identify the presence of viable microorganisms in a given sample as well as helps quantify them if present.[5] In general preparations, like *Kasaya* (decoction), *Ghrita* (ghee preparations) etc microorganisms are not acceptable as their presence indicate contamination by the particular organism. But as far as fermented products like *Aristas* are concerned, it is quite natural to detect the presence of these viable microorganisms as they are the sole of the entire formulation. Though organisms like Fungi, lacto bacilli are not acceptable as these organisms can make the end product into acetic acid. Comparison of CFUs of prepared and market samples is necessary as the presence of favorable microorganisms can give it a probiotic effect.

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Quick Response Code

https://doi.org/10.47070/ijapr.v13i1.3484

MATERIALS AND METHODS

Sample Selection

As per demand in the market based on an interview schedule among the company outlets, a total of three *Aristas-Abhayarista, Balarista* and *Jeerakarista* were selected for the study. Five market samples each of the corresponding *Aristas* were collected. Market sample analysis showed that *Abhayarista* as per the reference of *Ashtanga Hridaya* (AH) and *Bhaishajya*

Ratnavali (BR) was available for sale. Hence both references were taken into the study.

Sample Preparation

Materials used for the preparation is enlisted in Table 1. Genuine samples of raw drugs as per the reference were procured from authentic source. All the samples confirmed with corresponding API parameters. Genuine sample of jaggery within API limits was purchased from an organic shop in Thiruvananthapuram. The raw drugs were separately spread as a thin layer on a stainless steel tray and the foreign matter was hand sorted by visual inspection and later discarded if any. All raw drugs (except jaggery and *Dhataki* flowers) were washed separately, cleaned under tap water and dried in shade after spreading on separate stainless steel trays (75cm ×50cm). Once dried, they were transferred into separate glass bowls. *Dhataki pushpa* was dried in shade and kept in another glass bowl. Jaggery was pounded using roller stone by hand and resultant lumps were kept in another glass bowl.

For preparing *Kasaya* (decoction), the processed drugs were crushed separately on roller stone. From this required amount (as mentioned in corresponding table) was accurately weighed and transferred into a clean, dry stainless steel vessel of five liter capacity kept on gas stove. About one liter of water, measured using a measuring cylinder was transferred into the above stainless steel vessel containing crushed ingredients. The level of water was marked using measuring scale (This is the level up to which the whole *Kasaya* has to be reduced). Remaining three liter of water was transferred into the vessel. *Kasaya* was prepared on medium flame (*Mandagni)*, till it reached the marked level on the measuring scale.

Simultaneously, already processed raw drugs to be used as *Prakshepa dravya* were powdered separately using mixer grinder. The resultant powder was sieved using sieve no 44 and kept separately in glass bowls. From this required quantity of the powdered drugs was weighed on measuring balance. It was transferred into a mortar and triturated using a pestle to obtain homogenous mixture. The mouth of mortar was covered using cling film to avoid contact with moisture.

A clean porcelain jar of two liter capacity with lid was taken. It was made moisture free by exposing it to sunlight for two hours. Cow's ghee was smeared on interior using a hand glove followed by fumigation with five grams of *Guggulu. Guggulu* was made into a wick (*Varti*) using a ghee smeared cotton cloth. The lid of jar was kept closed for 10 minutes.

When *Kasaya* reduced to one liter, the vessel along with contents was removed from fire. It was filtered through double layered cotton cloth (25×25cm) into another clean stainless steel vessel of two liter capacity. To this filtrate accurately weighed, pounded jaggery was added in lukewarm state. It was stirred thoroughly using stainless steel ladle until jaggery completely dissolved in the *Kasaya*. It was left to cool to ambient temperature.

The mixture was filtered through double layered cotton cloth into the pre-processed porcelain jar. The homogenous mixture of *Prakshepa* in the mortar was added. Required quantity of *Dhataki pushpa* was crushed using mortar and pestle and added along with *Prakshepa*. The content was stirred to get uniform mixture. The lid of jar was screwed.

A cotton cloth, about one meter length and 15 cm breadth was smeared with paste of clay (Multani mitti) and water. The cloth was wrapped around the edges between mouth and lid of porcelain vessel in seven consecutive layers. When the mud plastering dried well, the jar was transferred into a thermo coal box to maintain a controlled temperature around 28 degrees Celsius. The box was fitted with a temperature controller to note the inside temperature. It was left undisturbed for a month.

After stipulated time, porcelain jar was taken out. Mud plastering was scraped off and lid was opened. The content was filtered through double layered cotton cloth of 25×25cm. Each of the *Aristas* was repeated for three times. The resultant products were measured using measuring cylinder and stored in a clean, dry, airtight amber colored glass bottle of one liter capacity. They were labeled for easy identification. All *Aristas* were prepared as per the above provided Standard Operating Procedure. Each of the *Aristas* was prepared thrice. However the differences in ingredients and quantity are enlisted below from Table 2-5.

Table 1: Materials required

Materials required	Quantity
Stainless steel tray	1
Stainless steel vessel	2
Roller stone	1
Mortar and pestle	1
Glass bowls	QS

***Abhayarista (AH)*[6]**

Table 2: Ingredients of *Abhayarista* (*AH*)

S.no	Name of drug	Botanical name	Part used	Proportion in *Yoga*	Amount taken
1	*Abhaya*	*Terminalia chebula* Retzius	Fruit rind	8 *Pala*	32g
2	*Amalaki*	*Emblica officinalis* Gatertn	Fruit rind	16 *Pala*	64g
3	*Kapithah*	*Feronia limonia* Linn	Fruit pulp	10 *Pala*	40g
4	*Visala*	*Citrullus colocynthis*	Root	*5 Pala*	20g
5	*Lodra*	*Symplocos racemosa* Roxb	Stem bark	2 *Pala*	8g
6	*Maricha*	*Piper nigrum* Linn	Fruit	2 *Pala*	8g
7	*Pippali*	*Piper longum*	Fruit	2 *Pala*	8g
8	*Vidanga*	*Embelia ribes* Burm	Fruit	2 *Pala*	8g
9	*Ela valuka*	*Prunus cerasus*	Seed	2 *Pala*	8g
10	*Dhataki*	*Woodfordia fruticosa* Kurz	Dried flower	16 *Pala*	64g
11	*Guda*			100 *Pala*	400g
12	Water for decoction			1000 (4 *Drona* reduced to 1 *Drona*	4000ml reduced to 1000ml

Prepared *Abhayarista* were named as H_1, H_2 and H_3 respectively and stored with label.

***Abhayarista (BR)*[7]**

Table 3: Ingredients of *Abhayarista* (*BR*)

S.no	Name of drug	Botanical name	Part used	Proportion in *Yoga*	Amount taken
1	*Abhaya*	*Terminalia chebula* Retzius	Fruit rind	100 *Pala*	400g
2	*Mridwika*	*Vitis vinifera*	Dried fruit	50 *Pala*	200g
3	*Vidana*	*Embelia ribes* Burm	Fruit	10 *Pala*	40g
4	*Madhuka kusuma*	*Madhuca indica* J.F.Gmel	Flower	10 *Pala*	40g
5	*Swadamstra*	*Tribulus terrestris* Linn	Fruit	1 *Pala*	4g
6	*Trivrit*	*Operculina turpethum* Linn Silva Manso	Root	1 *Pala*	4g
7	*Dhanyaka*	*Coriandrum sativum*	Fruit	1 *Pala*	4g
8	*Indravaruni*	*Citrullus colocynthis*	Root	1 *Pala*	4g
9	*Cavya*	*Piper chaba* Hunter	Stem	1 *Pala*	4g
10	*Misreya*	*Foeniculum vulgare* Mill	Fruit	1 *Pala*	4g
11	*Sunti*	*Zingiber officinale* Roscoe	Rhizome	1 *Pala*	4g
12	*Danti*	*Baliospermum montanum* Muell	Root	1 *Pala*	4g
13	*Mocarasa*	*Salmalia malabarica* Schott & Endl	Exudate	1 *Pala*	4g
14	*Dhataki*	*Woodfordia fruticosa* Kurz	Dried flower	1 *Pala*	4g
15	*Guda*			100 *Pala*	400g
16	Water for decoction			1000 (4 *Drona* reduced to 1 *Drona*)	4000ml reduced to 1000ml

They were named as A_1, A_2, and A_3 respectively and stored as specified with the label.

Jeerakarista (BR) [8,9,10]

Table 4: Ingredients of *Jeerakarista (BR)*

They were named J_1, J_2 and J_3 and stored after proper labeling.

S.no	Name of drug	Botanical name	Part used	Proportion in *Yoga*	Amount taken
1	*Jeeraka*	*Cuminum cyminum* Linn	Fruit	200 *Pala*	800g
2	*Sunti*	*Zingiber officinale* Roscoe	Rhizome	2 *Pala*	8g
3	*Jati phala*	*Myristica fragrans* Houtt	Seed	1 *Pala*	4g
4	*Musta*	*Cyperus rotundus* Linn	Rhizome	1 *Pala*	4g
5	*Twak*	*Cinnamomum zeylanicum* Linn	Stem bark	1 *Pala*	4g
6	*Ela*	*Elettaria cardamomum* Linn	Seed	1 *Pala*	4g
7	*Patra*	*Cinnamomum tamala* Nees	Leaf	1 *Pala*	4g
8	*Nagakesara*	*Mesua ferrea* Linn	Stamen	1 *Pala*	4g
9	*Yavani*	*Trachyspermum ammi* Linn	Fruit	1 *Pala*	4g
10	*Kankola*	*Piper cubeba* Linn	Fruit	1 *Pala*	4g
11	*Lavanga*	*Syzygium aromaticum* Linn	Flower bud	1 *Pala*	4g
12	*Dhataki*	*Woodfordia fruticosa* Kurz	Dried flower	16 *Pala*	64g
13	*Guda*			300 *Pala*	1200g
14	Water for decoction			1000 (4 *Drona* reduced To 1 *Drona*	4000ml reduced to 1000ml

Balarista (BR)[11,12,13]

Table 5: Ingredients of *Balarista* (*BR*)

After the process they were named as B_1, B_2 and B_3 respectively and stored after proper labelling.

S. no	Name of drug	Botanical name	Part used	Proportion in *Yoga*	Amount taken
1	*Bala*	*Sida cordifolia* Linn	Root	100 *Pala*	400g
2	*Aswagandha*	*Withania somnifera*	Root	100 *Pala*	400g
3	*Kshira vidari*	*Ipomea digitata* Linn	Sub.root	2 *Pala*	8g
4	*Panchangula*	*Ricinus communis* Linn	Root	2 *Pala*	8g
5	*Rasna*	*Alpinia galangal*	Rhizome	1 *Pala*	4g
6	*Sukshma ela*	*Elattaria cadamomum* Linn	Seed	1 *Pala*	4g
7	*Prasarini*	*Paederia foetida* Linn	Whole plant	1 *Pala*	4g
8	*Lavanga*	*Syzgyium aromaticum* Linn	Flower bud	1 *Pala*	4g
9	*Usira*	*Vetiveria zizanioides* Linn Nash	Root	1 *Pala*	4g
10	*Gokshura*	*Tribulus terrestris* Linn	Fruit	1 *Pala*	4g
11	*Dhataki*	*Woodfordia fruticosa* Kurz	Dried flower	16 *Pala*	64g
12	*Guda*			300 *Pala*	1200g
13	Water for decoction			1000 (4 *Drona* reduced to 1 *Drona*)	4000ml reduced to 1000ml

Market sample collection

Five market samples each of *Abhayarista*, *Balarista* and *Jeerakarista* produced by a manufacturing company in Kerala and *having* same month of manufacture as that of the corresponding prepared *Aristas* were collected from the company outlets. It was noticed that only two samples of *Abhayarista* as per reference of *Bhaishajya Ratnavali* was available in the Kerala market. Hence the total number of market sample to be analyzed is 17. Market samples of *Abhayarista* (AH) were labeled as M1, M2, M3, M4 and M5, samples of *Abhayarista* (BR) named as P1 and P2, *Balarista* as N1, N2, N3, N4 and N5, *Jeerakarista* as L1, L2, L3, L4 and L5.

CFU analysis

1. Enumeration of Colony Forming Units[14,15]

Materials required

- **Mueller hinton agar**

  The Mueller Hinton Agar media was prepared by dissolving 38g MHA in 1000ml distilled water and sterilized by autoclaving at 121°C 15lbs for 15mins. Afterwards the media was allowed to cool to 50°C and were poured on to pre-sterilized, pre-labelled petri plates and were allowed to solidify inside laminar airflow chamber with flow and UV.

- **Procedure**

  The whole procedure was conducted in a laminar air flow hood. 20µL from each samples were dropped to respective petri plates and were swabbed on to Mueller Hinton agar. The plates were incubated at 37°C for 24 hours in a microbiological incubator. After incubation the plates were observed for colony forming units (CFUs). The CFUs were counted using a Digital Colony counter and were expressed as CFUs/mL.

RESULTS AND DISCUSSION

Results of CFU of prepared *Aristas* are summarized in Table 6.

Table 6: CFU of prepared *Aristas*

Sample	No of colony counted	CFU/mL
A1	127	$6.35*10^3$
A2	5	$0.25*10^3$
A3	0	-
B1	2	$0.1*10^3$
B2	0	-
B3	TNTC	-
H1	19	$0.95*10^3$
H2	38	$1.9*10^3$
H3	5	$0.25*10^3$
J1	93	$4.65*10^3$
J2	39	$1.95*10^3$
J3	88	$4.4*10^3$

TNTC- Too Numerous To Count

Results of CFU of market samples of the selected *Aristas*, *Jeerakarista* (L1 to L5), *Abhayarista (*AH) (M1 to M5), *Balarista* (N1 to N5) and *Abhayarista (*BR)(P1 and P2) are summarized in Table 7.

Table 7: CFU of market samples

Sample	No of colony counted	CFU/mL
L1	98	$49*10^2$
L2	22	$11*10^2$
L3	3	$1.5*10^2$
L4	TNTC	-
L5	92	$46*10^2$
M1	1	$0.5*10^2$
M2	2	$1*10^2$
M3	1	$0.5*10^2$
M4	2	$1*10^2$
M5	1	$0.5*10^2$
N1	118	$59*10^2$

N2	31	-
N3	19	-
N4	55	-
N5	15	-
P1	83	-
P2	849	-

Statistical Analysis

Prepared samples and market samples were compared using one sample t test as summarized in Table 8. A calculated P value less than 0.05 is considered statistically significant. All the analyses were carried out with the help of commercially available statistical package SPSS v.23 for WINDOWS.

Table 8: Statistical analysis of prepared and market samples

	Prepared sample	Market sample	t value	P value
Colony forming units	44.000 ± 41.525	P1: 83	-0.939	0.447NS
		P2: 849	-19.386	0.003**
	20.667 ± 9.563	M1: 1	2.057	0.176NS
		M2: 2	1.952	0.190NS
		M3: 1	2.057	0.176NS
		M4: 2	1.952	0.190NS
		M5: 1	2.057	0.176NS
	73.333 ± 17.227	L1: 98	-1.432	0.289NS
		L2: 22	2.980	0.097NS
		L3: 3	4.083	0.055NS
		L4: 1000	-53.791	<0.001**
		L5: 92	-1.084	0.392NS
	334.000 ± 333.000	N1: 118	0.649	0.583NS
		N2: 31	0.910	0.459NS
		N3: 19	0.946	0.444NS
		N4: 55	0.838	0.490NS
		N5: 15	0.958	0.439NS

****: Significant at 1% (P value < 0.01); NS: Not Significant at 5% (P value > 0.05)**

From the above results, a one-sample t-test showed that the colony forming unit measurements based on P2 and L4 are significantly different from those of the prepared sample (P value< 0.01), while the measurements for P1, M1, M2, M3, M4, M5, L1, L2, L3, L5, N1, N2, N3, N4 and N5 are the same as those of the prepared sample (P value > 0.05).

Ingredients of *Abhayarista* (AH)

Abhaya ***Kapithah*** ***Visala*** ***Pippali***

Vidanga ***Elavaluka*** ***Lodra*** ***Marica***

Dhataki ***Guda***

Figure 1: Raw drugs of *Abhayarista* (AH)

Ingredients of *Abhayarista* (BR)

Abhaya ***Vidanga*** ***Draksha*** ***Madhuka pushpa***

Guda ***Gokshura*** ***Dhanyaka*** ***Trivrit***

Dhataki ***Chavya*** ***Visala*** ***Shunti***

Danti *Mocarasa* *Misreya*

Figure 2: Raw drugs of *Abhayarista* (BR)

Ingredients of *Balarista* (BR)

Bala *Aswagandha* *Guda* *Rasna*

Dhataki *Kshira vidari* *Eranda* *Prasarini*

Lavanga *Gokshura* *Ela* *Usira*

Figure 3. Raw drugs of *Balarista*

Ingredients of *Jeerakarista* (BR)

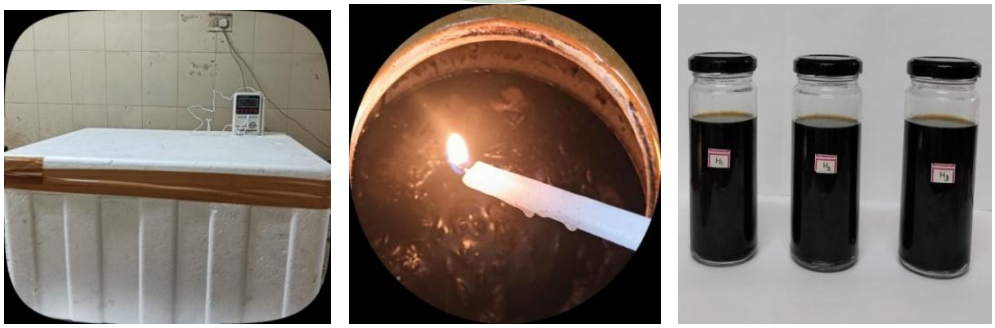
Jeeraka *Guda* *Dhataki* *Shunti*

Jatiphala ***Musta*** ***Twak*** ***Patra***

Nagakesara ***Yavani*** ***Maricha*** ***Lavanga*** ***Ela***

Figure 4: Raw drugs of *Jeerakarista*

Preparation of *Kasaya* **Mixing of *Prakshepa* and *Dhataki*** ***Sandhi bandhana* of *Sandhana patra***

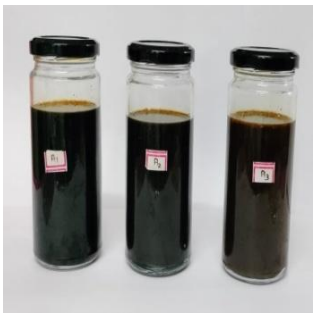


Kept for *Sandhana* in Thermocol box **Burning candle test** **Final products H1, H2, H3**

Figure 5: Preparation of *Abhayarista* (AH)

Preparation of *Kasaya* **Mixing of *Prakshepa* and *Dhataki*** ***Sandhibandhana* of *Sandhana patra***





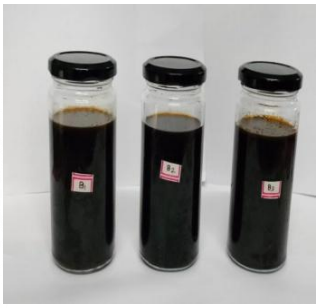
Kept for *Sandhana* in Thermocol box **Burning candle test** **Final products A1, A2, A3**

Figure 6: Preparation of *Abhayarista* (BR)



Preparation of *Kasaya* **Mixing of *Prakshepa* and *Dhataki*** ***Sandhibandhana* of *Sandhana patra***





Kept for *Sandhana* in Thermocol box **Burning candle test** **Final products B1, B2, B3**

Figure 7: Preparation of *Balarista* (BR)



Preparation of *Kasaya* **Mixing of *Prakshepa* and *Dhataki*** ***Sandhi bandhana* of *Sandhana patra***



Kept for *Sandhana* in Thermocoal box **Burning candle test** **Final products J1, J2, J3.**

Figure 8: Preparation of *Jeerakarista* (BR)

Market Samples of Selected *Aristas*

Market samples of *Abhayarista* (AH)

Market samples of *Abhayarista* (BR)

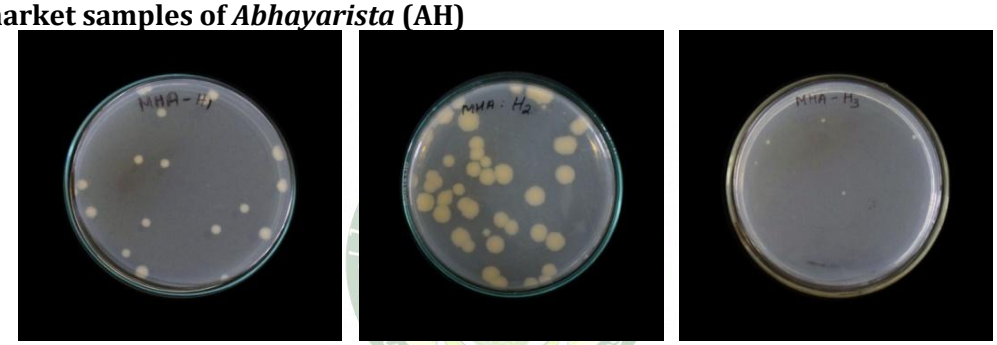
Market samples of *Jeerakarista*

Market samples of *Balarista*

Figure 9: Market samples of selected *Aristas*

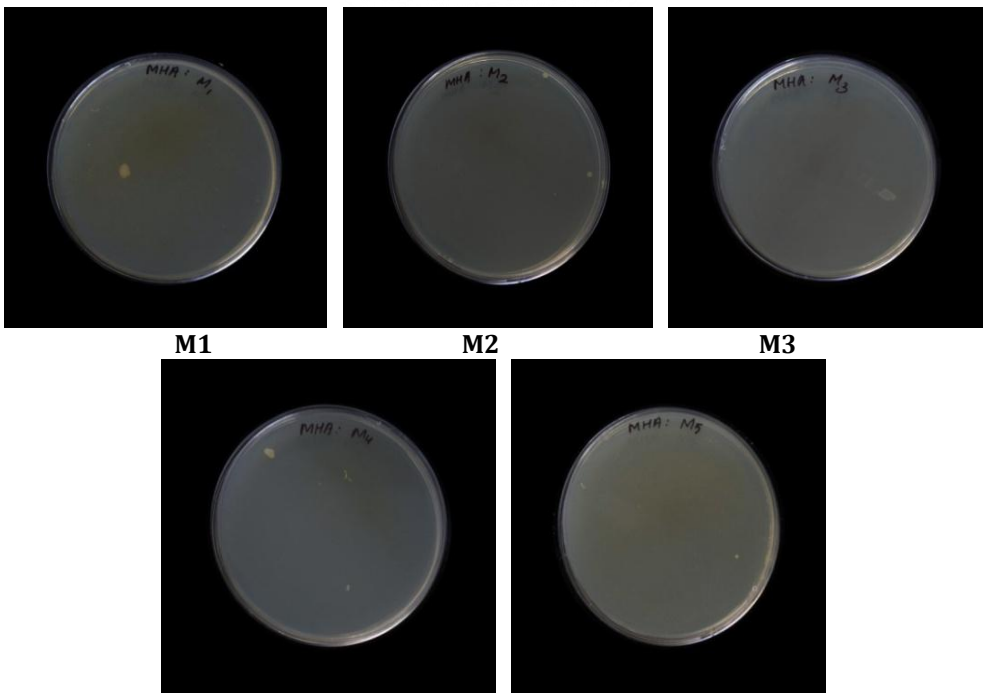
Colony Forming Units

Prepared and market samples of *Abhayarista* (AH)



H1 **H2** **H3**

Figure 10: CFU of prepared samples of *Abhayarista* (AH) - H1, H2, H3.

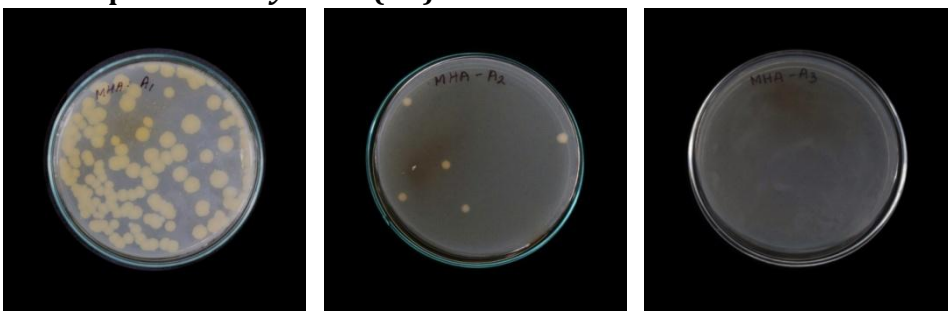


M1 **M2** **M3**

M4 **M5**

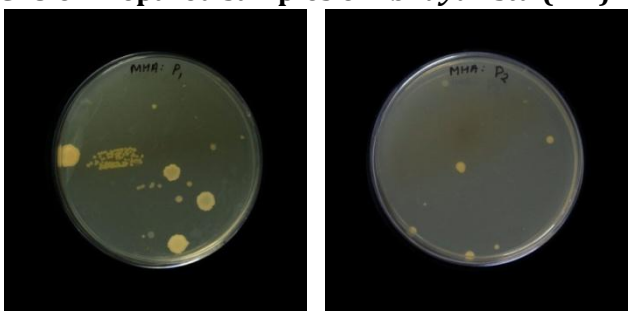
Figure 11: CFU of Market samples of *Abhayarista* (AH) - M1, M2, M3, M4, and M5.

Prepared and market samples of *Abhayarista* (BR)



A1 **A2** **A3**

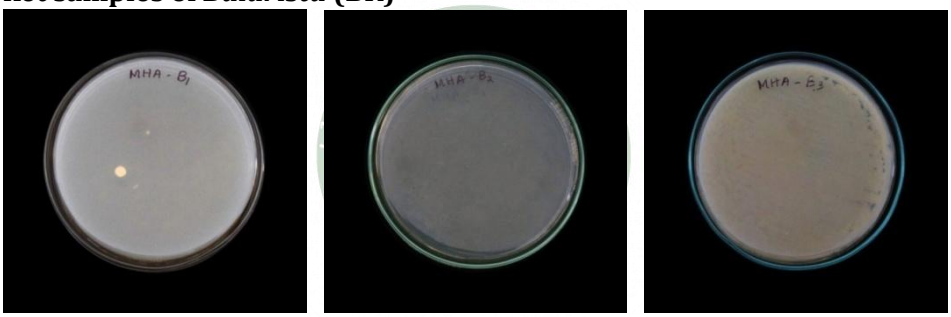
Figure 12: CFU of Prepared samples of *Abhayarista* (B R) - A1, A2, A3.



P1 **P2**

Figure 13: CFU of Market samples of *Abhayarista* (B R) - P1 and P2.

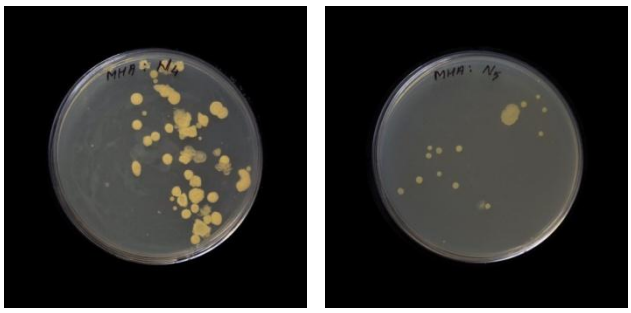
Prepared and market samples of *Balarista* (BR)



B1 **B4** **B3**

Figure 14: CFU of Prepared samples of *Balarista* (B R) - B1, B2, B3.

N1 **N2** **N3**



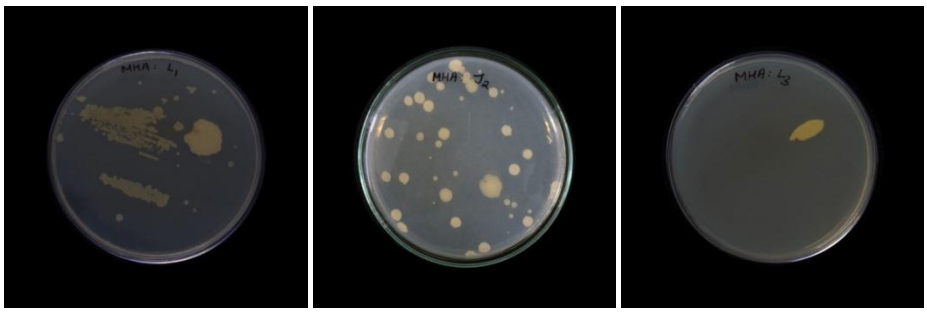
N4 **N5**

Figure 15: CFU of Market samples of *Balarista* (B R)-N1, N2, N3, N4 and N5.

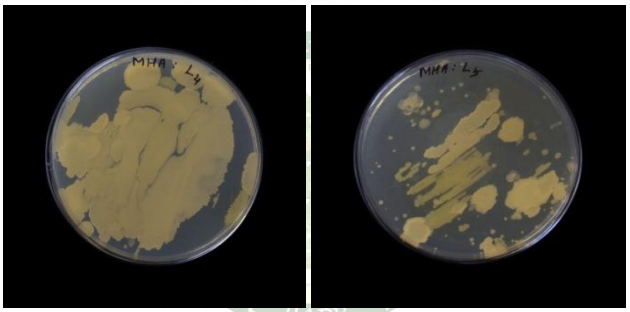
Prepared and market samples of *Jeerakarista* (BR)

J1 **J2** **J3**

Figure 16: CFU of Prepared samples of *Jeerakarista* (B R) - J1, J2, J3.



L1 **L2** **L3**



L4 **L5**

Figure 17: CFU of Market samples of *Jeerakarista* (B R) - L1, L2, L3, L4 and L5.

DISCUSSION

CFU's are used to estimate the number of viable microorganisms in a sample. Viable cells are those that are capable of dividing and forming colonies. It is not a confirmatory test for specific identity of organisms. They are just qualitative tests.

CONCLUSION

Prepared samples are having more number of microbial colonies compared to the market samples. This might be due to the addition of preservatives in the market samples. Preservatives generally arrest the microbial growth by making the environment unfavourable for these organisms to thrive. Some of the market samples have comparatively more colonies. TNTC (Too Numerous to Count) was observed among prepared (B3) and market samples (L4). This indicates that the concentration of microorganisms in those samples are very high than the limit of detection (i.e., 1000). Prepared samples A3 and B2 have CFU count zero. This means the colonies are below the detection limit. The presence of colonies indicates that there are potent viable organisms that can mediate the fermentation process again in the samples. It indicates the probiotic effect too. The probiotic effect in prepared samples is more when compared to that of market samples indicating some compromise might have been made in the marketed formulation.

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Cite this article as:
Shilpa S, Rajam R. Comparison of Colony Forming Units (CFU) Between Prepared and Market Samples of Selected Aristas in the Kerala Market. International Journal of Ayurveda and Pharma Research. 2025;13(1):59-72.
https://doi.org/10.47070/ijapr.v13i1.3484
Source of support: Nil, Conflict of interest: None Declared

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