

Benchmarking the Quality of Ashwagandharishta: Comparative Evaluation of Three Marketed Brands

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Abstract

This study aims to benchmark the quality of Ashwagandharishta by conducting a comparative quality evaluation of three marketed brands. The objectives are to assess various quality parameters to determine the overall quality and efficacy of each brand. Three brands of Ashwagandharishta were procured from the market and subjected to quality assessment as per the various methods prescribed in the standard texts of Pharmacopoeias. The analysis revealed that all three brands adhered to the quality standards prescribed in Ayurvedic pharmacopeia. Organoleptic properties were consistent across the brands, with minor variations in color. Physicochemical parameters, including pH and specific gravity, were within acceptable ranges, with no brand showing any alcohol content. Phytochemical, microbial contamination and heavy metal concentration analysis indicated compliance with the prescribed standards. The findings underscore the importance of consistent quality control measures in the production of Ayurvedic formulations. This comparative evaluation demonstrates that while all three brands of Ashwagandharishta meet essential quality parameters, variations in specific attributes can impact their therapeutic effectiveness. Ensuring stringent quality control is crucial for maintaining the efficacy and consumer trust in Ayurvedic products. This study provides valuable insights for both manufacturers and consumers, promoting informed decision-making.

Key words: Ashwagandharishta, Quality assessment, Organoleptic evaluation, Physicochemical evaluation, Pharmaceutical evaluation, Heavy metal content, Microbial contamination

Ashwagandharishta is a traditional Ayurvedic herbal formulation primarily derived from the roots of Ashwagandha (*Withania somnifera*). Ashwagandharishta holds significant importance in Ayurvedic medicine due to its comprehensive health benefits. It is considered a Rasayana, which means it has rejuvenating properties that promote longevity and overall health. Its adaptogenic nature makes it particularly valuable in managing stress, anxiety, and mental fatigue. Additionally, it plays a crucial role in improving physical strength, stamina, and immunity, making it a holistic health tonic.

Ashwagandharishta is prepared through a meticulous fermentation process that involves several herbs and natural ingredients. The primary component is Ashwagandha root, which is known for its potent therapeutic properties.

Other key ingredients include:

Manjishtha (*Rubia cordifolia*): Known for its blood-purifying properties.

Haritaki (*Terminalia chebula*): Helps in digestion and detoxification.

Amalaki (*Emblica officinalis*): Rich in Vitamin C, it boosts immunity.

Musta (*Cyperus rotundus*): Used for its anti-inflammatory and digestive benefits.

Vidanga (*Embelia ribes*): Acts as an antimicrobial agent.

Jaggery: Used as a fermenting agent and provides a natural sweetness.

Water: The base for the fermentation process.

These ingredients are combined and left to ferment for a specific period, resulting in a bioactive tonic that maximizes the therapeutic potential of the herbs. The Ashwagandharishta is used for various ailments such as:

Stress and anxiety relief: The adaptogenic properties of Ashwagandha help the body cope with stress and reduce anxiety levels by balancing the Vata and Pitta doshas [1].

Immune system enhancement: The presence of Amalaki, with its high Vitamin C content, significantly contributes to this benefit [2].

Improved cognitive function: It acts as a brain tonic, improving mental clarity and reducing symptoms associated with cognitive decline [3].

Physical strength and stamina: It helps in increasing physical strength and stamina. It is particularly beneficial for individuals recovering from illnesses or those experiencing general weakness [4].

Digestive health: Ingredients like Haritaki and Musta support the digestive system, helping to alleviate digestive disorders and promoting better nutrient absorption [5].

Anti-inflammatory and antioxidant properties: The formulation has significant anti-inflammatory and antioxidant effects, which help in reducing inflammation and oxidative stress in the body [5].

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Hormonal balance: Ashwagandharishta supports hormonal balance, particularly in women. It helps in managing menstrual irregularities and symptoms of menopause by supporting endocrine function [6].

According to ayurvedic pharmacopoeia of India, the typical composition of Ashwagandharishta consists of around 7 medicinal herbs a primary ingredient with Honey. The composition of the formulation is mentioned in (Table 1).

Table 1 Composition of Ashwagandharishta according to Ayurvedic Pharmacopoeia of India

Name of drug	Latin name
Ashvagandha API	<i>Withania somnifera</i>
Musali API	<i>Chlorophytum tuberosum</i>
Manjistha API	<i>Rubia cordifolia</i>
Haritaki API	<i>Terminalia chebula</i>
Haridra API	<i>Curcuma longa</i>
Daruharidra API	<i>Berberis aristata</i>
Madhuka (Yashti API)	<i>Glycyrrhiza glabra</i>
Rasna API	<i>Pluchea lanceolata</i>
Vidari API	<i>Pueraria tuberosa</i>
Pirtha (Arjuna API)	<i>Terminalia arjuna</i>
Mustaka (Musta API)	<i>Cyperus rotundus</i>
Trivrit API	<i>Ipomoea turpethum</i>
Ananta (Shveta sariva API)	<i>Hemidesmus indicus</i>

Shyama (Krishna Sariva)	<i>Cryptolepis buchanani</i>
Shveta Chandana API	<i>Santalum album</i>
Rakta Chandana API	<i>Pterocarpus santalinus</i>
Vaca API	<i>Acorus calamus</i>
Citraka API	<i>Plumbago zeylanica</i>
Jala for decoction	Water
Prakshepa Dravyas	
Makshika (Madhu API)	Honey
Dhataki API	<i>Woodfordia fruticosa</i>
Shunthi API	<i>Zingiber officinale</i>
Marica API	<i>Piper nigrum</i>
Pippali API	<i>Piper longum</i>
Tvak API	<i>Cinnamomum zeylanicum</i>
Ela (Sukshmaila API)	<i>Elettaria cardamomum</i>
Patra (Tejapatra API)	<i>Cinnamomum tamala</i>
Priyangu API	<i>Callicarpa macrophylla</i>
Nagakeshara API	<i>Mesua ferrea</i>

MATERIALS AND METHODS

Procurement of samples

All brands of the Ashwagandharishta were procured from the local market from the registered ayurvedic pharmacy. The following marketed Ashwagandharishta preparations were used in the present study (Table 2) [5-14].

Table 2 Details of formulations procured from the market for conducting the proposed study

Brand	Brand name	Code assigned	Net Volume/Quantity	Batch No.	Manufacture date	Expiry date
Brand A	Baidyanath	HI/CL/01/BD	450 ml	827	Mar-22	Feb-32
Brand B	Dabur	HI/CL/01/DB	450 ml	BD00605	May-22	Apr-32
Brand C	Dindayal	HI/CL/01/DD	450 ml	BASA2112	Apr-22	Mar-32

Organoleptic evaluation

All the organoleptic properties viz. color, odor and taste were performed as per standard procedure and noted down.

Determination of pH value

A 5g sample was immersed in 100 ml of water, covered, and left at room temperature for 24 hours. The supernatant was decanted, and the pH was measured using a calibrated digital pH meter.

Determination of weight per milliliters

Formula for calculation

$$\text{Weight per ml} = \frac{W_3 - W_1}{\text{Capacity of RD Bottle ml}} \text{ mg}$$

Determination of specific gravity at 25°C

Formula for calculation

$$\text{Specific gravity} = \frac{\text{Weight of sample}}{\text{Weight of water}} = \frac{W_3 - W_1}{W_2 - W_1}$$

Determination of viscosity

It is determined with the help of Ostwald Viscometer.

Formula for calculation

$$\eta_2 = \frac{\eta_1 \times \rho_2 t_2}{\rho_1 t_1}$$

Determination of relative surface tension

It is determined with the help of Stalagmometer.

Formula for calculation

$$\gamma_2 = \frac{\rho_2 n_1}{\rho_1 n_2} \times \gamma_1$$

Determination of total solids

Accurately transfer 50 ml of clear Asava/Arishta to a pre-weighed evaporating dish. Evaporate to dryness on a water

bath, then dry at 105°C for three hours. Cool in a desiccator for 30 minutes, then weigh immediately. Ensure the residue weight meets individual monograph requirements.

Formula for calculation

$$\% \text{ Residue} = W_2 - W_1$$

Determination of alcohol content

The ethanol content of a liquid is expressed as the number of volumes of ethanol contained in 100 volumes of the liquid, the volumes being measured at 24.9° to 25.1°. This is known as the "percentage of ethanol by volume". The Total Alcohol content of the formulation was determined by Method-III prescribed in Ayurvedic Pharmacopoeia of India.

Test for methanol

The Esterification test involves adding salicylic acid crystals and a few drops of concentrated H₂SO₄ to ½ ml of the sample. Upon gentle heating, a characteristic Wintergreen oil odor indicates methyl salicylate formation.

Determination of total sugar, reducing sugar and non-reducing sugar

The method of Lane and Eyonon by reduction of Fehling's solution is the most generally applied volumetric method, the use of methylene blue as an internal indicator increasing the accuracy of the process.

Formula for calculation

$$\% \text{ Total sugars (as invert sugars)} = \frac{0.5 \times V_1 \times V_2}{V_3 \times W}$$

$$\% \text{ Total reducing sugars} = \frac{0.25 \times V_1 \times V_2}{V_4 \times W}$$

Phytochemical evaluation

The aqueous and alcoholic extracts of the respective formulations were prepared and were subjected to preliminary phytochemical screening. These tests reveal the presence of

various bioactive secondary metabolites which might be responsible for their medicinal attributes. Methods for preliminary qualitative phytochemical tests of the plant extracts are given below in the (Table 3).

Table 3 Preliminary phytochemical tests for plant extracts

S. No.	Phyto-constituents	Name of Tests	Procedure	Observation
1.	Alkaloids	Mayer's test	2 ml extract + few drops of HCl + Mayer's reagent	Cream Precipitation
		Hager's test	2 ml extract + few drops of HCl + Hager's reagent	Yellow Precipitation
		Wagner's test	2 ml extract + few drops of HCl + Wagner's reagent	Reddish brown color
2.	Carbohydrates	Molisch's test	2 ml extract + 2 Drops of Molisch reagent + few drops of Conc. H ₂ SO ₄	Violet or Reddish color
3.	Reducing sugars	Fehling's test	1 ml extract + 1 ml Fehling Solution (A and B)	First a Yellow and then Brick Red Precipitation
4.	Flavonoids	Alkaline reagent test	2 ml extract + few drops of 40% NaOH solution	Intense yellow color forms which become colorless on addition of dil. acid
		Lead acetate test	2 ml extract + few drops of Lead Acetate solution	Yellow precipitation
5.	Saponins	Foam test	2 ml extract + 4 ml distilled H ₂ O Mix well and shake vigorously	Foam formation
6.	Tannins	Braymer's test	2 ml extract + 2 ml H ₂ O + 2-3 drops of 5% FeCl ₃	Black green or bluish color
7.	Steroids	Salkowski's test	2 ml extract + 2 ml Chloroform + 2 ml Conc. H ₂ SO ₄	Chloroform layer appears red and acid layer shows greenish-yellow fluorescence
8.	Proteins	Millon's test	3 ml extract + 5 ml Millon's reagent	White precipitate which turns brick red on warming
9.	Glycosides	Keller Killiani's test	2 ml extract + Glacial acetic Acid + 1 drop of 5% FeCl ₃ + Conc. H ₂ SO ₄	Reddish brown color appears at the junction of 2 layers and upper layer appears bluish green
10.	Phenols	-	2-3 ml of extract + few drops of 5% FeCl ₃ solution	Deep blue-black color
			2-3 ml of extract + few drops of Lead Acetate solution	White precipitate
11.	Amino acids	Ninhydrin test	3 ml of extract + 3 drops of 5% Ninhydrin solution Keep in boiling water bath for 10 min.	Purple or bluish color appears
12.	Terpenoids	Copper Acetate test	2 ml extract dissolved in water + 3-4 drops of Copper Acetate solution	Emerald green color

Determination of heavy metals (Lead and Cadmium)

Method (Direct Calibration Method)

Three reference solutions of the element being examined having different concentrations were prepared covering the range recommended by the instrument manufacturer. Separately the corresponding reagents were added for the test solution and the blank solution was prepared with the corresponding reagents. The absorbance of the blank solution and each reference solution were measured separately, and the readings were recorded. A calibration curve was prepared. The

test solution was prepared as per the method prescribed in the Ayurvedic Pharmacopoeia of India.

Sample analysis

The analysis of the digested samples was carried out using an atomic absorption spectrophotometer (Agilent Technologies VARIAN Spectro AA220FS Atomic Absorption Spectrophotometer) for lead and cadmium. The instrumental conditions for lead and cadmium analysis are depicted in (Table 4).

Table 4 Instrumental conditions for analysis of lead and cadmium

Parameters	Lead (Pb)	Cadmium (Cd)
Wavelength (nm)	217	228.8
Slit width (nm)	1.0	0.5
Light Source	Hollow Cathode Lamp	Hollow Cathode Lamp
Flame type	Air/C ₂ H ₂	Air/C ₂ H ₂
Current (mA)	5	4
AAS Technique	Flame	Flame
Flame emission wavelength (nm)	405.8	326.1

Microbial limit test

1. Pretreat the sample of the product being examined as described in the method prescribed in Pharmacopoeia.

2. Plate count

For bacteria

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of liquified casein soyabean digest agar (SCA) at not more than 45°. Prepare at least two such Petri dishes using the same dilution and incubate at 30° to 35° for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

For fungi

Proceed as described in the test for bacteria but use Sabouraud dextrose agar (SDA) with antibiotics in place of

casein soyabean digest agar and incubate the plates at 20° to 25° for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies. The standard values for microbial contamination limit are represented in (Table 5).

Table 5 Microbial contamination limits

Parameters	Permissible limits
Total microbial plate count (TPC)	10 ⁵ /g
Total yeast and mould	10 ³ /g

RESULTS AND DISCUSSION

The results for the evaluation of various quality control parameters including organoleptic, physicochemical, pharmaceutical, phytochemical, microbial contamination and heavy metal analysis are depicted in (Table 6).

Table 6 Results of assessment of various quality control parameters for different brands of Ashwagandharishta

S. No.	Properties	Dabur	Baidyanath	Dindayal	Standard (API-II Vol-2)
1.	Description	Clear without frothing	Clear without frothing	Clear without frothing	Clear without frothing
2.	Color	Dark brown	Light brown	Dark brown	Dark brown
3.	Odor	Pleasant	Pleasant	Pleasant	
4.	Taste	Astringent	Astringent	Astringent	Astringent
5.	pH	4.3	3.6	3.9	3.5-4.5
6.	Weight (mg) per ml	1.033	1.072	1.105	-
7.	Specific gravity at 25°C	1.063	1.106	1.149	1.05-1.20
8.	Total solids (% w/v)	12.40%	17.90%	26.60%	NLT 18.5% w/v
9.	Viscosity (cp)	1.255	1.310	1.987	-
10.	Surface tension (dynes/cm)	46.06	58.65	56.90	-
11.	Total alcohol content (% v/v)	≈0%	≈0%	≈0%	5-10% v/v
12.	Test for methanol (Esterification test)	Negative	Negative	Negative	Negative
13.	Total sugar	26.15%	20.61%	22.97%	-
14.	Reducing sugar	25.48%	20.03%	22.32%	NLT 13% w/v
15.	Non-reducing sugar	0.67%	0.58%	0.65%	NMT 0.7% w/v
16.	Heavy metal concentration (ppm)				
a.	Lead	0.156	0.180	0.179	10 ppm
b.	Cadmium	0.010	0.024	0.018	0.3 ppm
17.	Microbial contamination				
a.	Total fungal count	<1	<1	<1	10 ³ CFU/g
b.	Total bacterial count	98	1	<1	10 ⁵ CFU/g

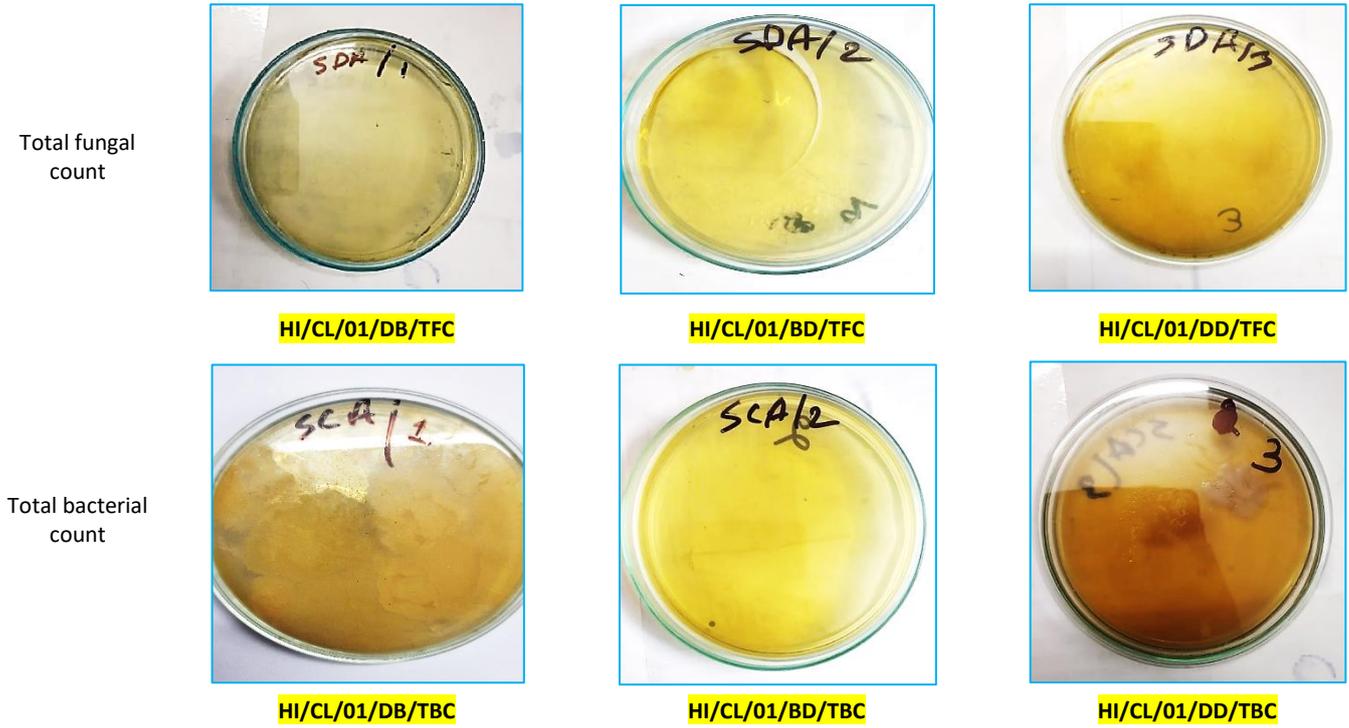
Table 7 Phytochemical Screening of Ashwagandharishta

S. No.	Phyto-constituent	Name of tests	Dabur	Baidyanath	Dindayal
1.	Alkaloids	Hager's test	-	-	-
		Wagner's test	-	-	-
		Mayer's test	-	-	-
2.	Glycosides	Keller Killani's test	+	+	+
		Molisch's test	+	+	+
3.	Carbohydrates	Fehling's test	+	+	+
4.	Reducing Sugars	Fehling's test	+	+	+
		Biuret's test	-	-	-
5.	Proteins	Millon's test	-	-	-
		Ninhydrin's test	+	+	+
6.	Amino Acids	Salkowski's test	+	+	+
7.	Steroids	Alkaline reagent test	-	-	-
		Lead acetate test	-	-	-
8.	Flavonoids	Copper acetate test	+	+	+
		Ferric chloride test	+	+	+
9.	Terpenoids	Foam test	-	-	-
10.	Tannins	Ferric chloride test	-	-	-
		Lead acetate test	-	-	-
11.	Saponins				
12.	Phenols	Ferric chloride test	-	-	-
		Lead acetate test	-	-	-

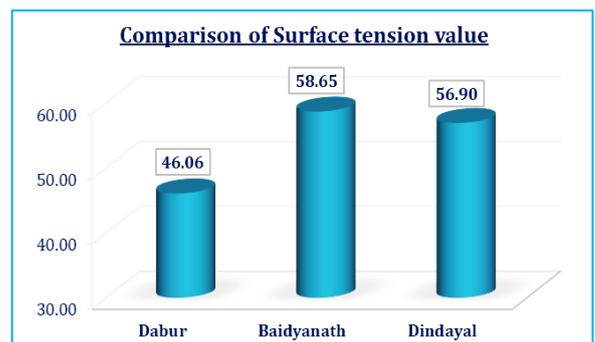
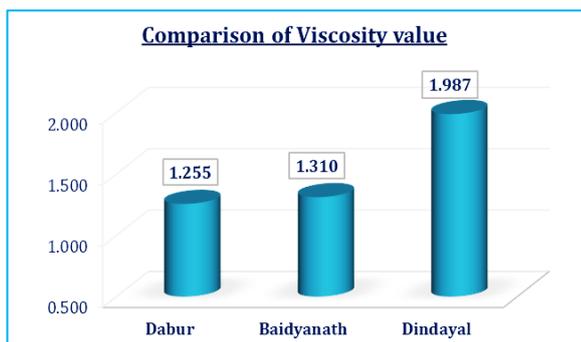
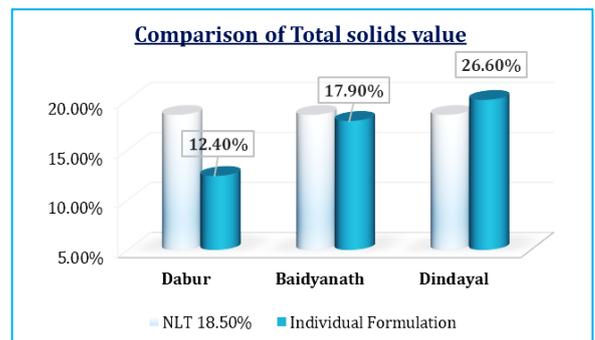
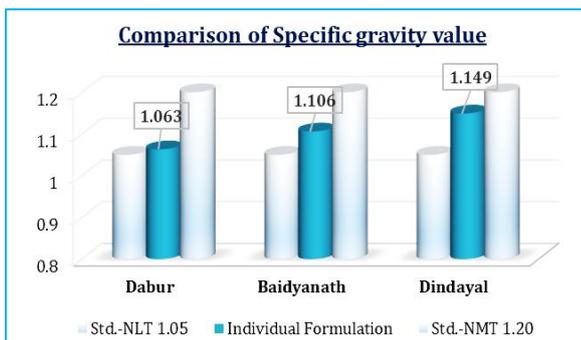
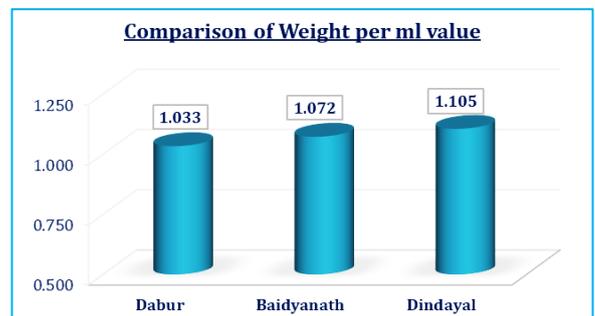
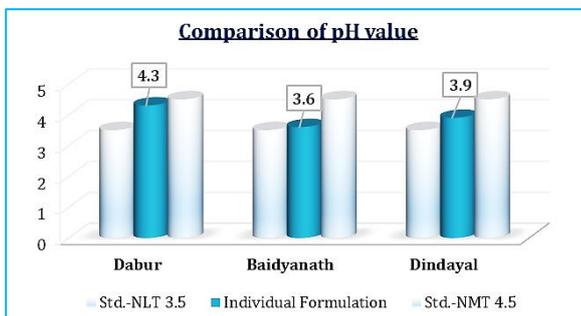
The following table represents that whether the results of evaluation parameters are complying with the standard value prescribed in the Ayurvedic Pharmacopoeia or not. Those

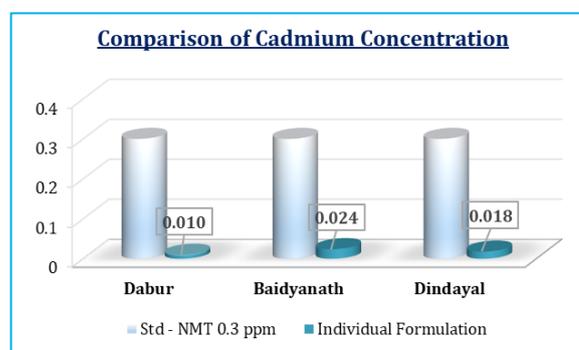
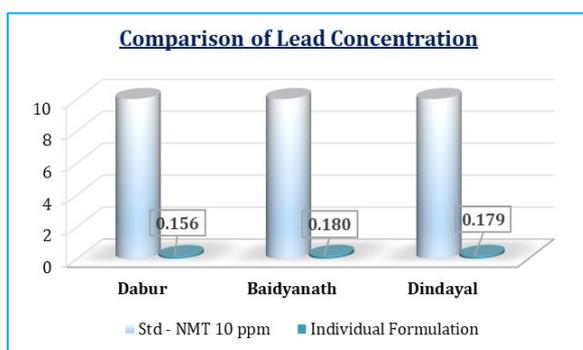
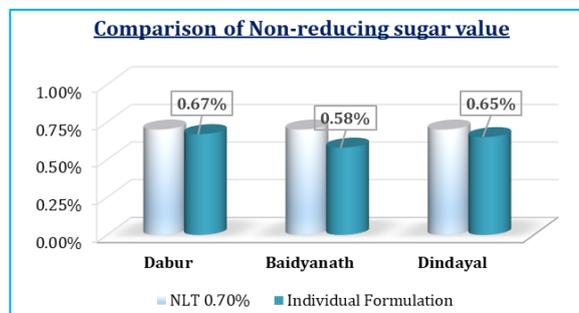
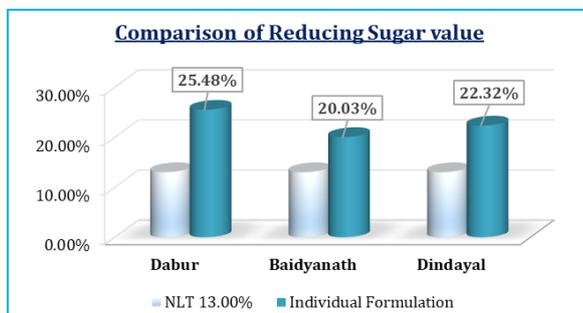
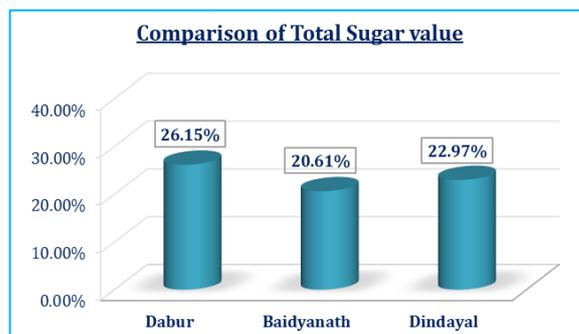
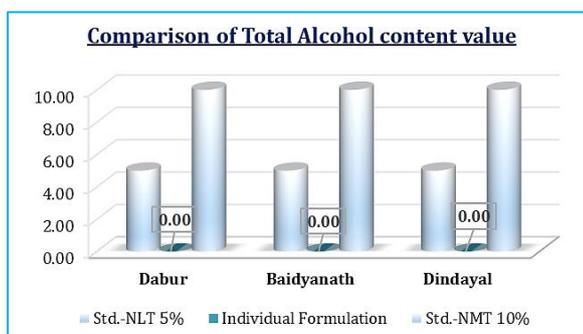
complying with the standard value are indicated with “C” and those that are not complying with the standard value are indicated with “NC”.

Table 8 Pictorial representation of results for Microbial Limit test of Ashwagandharishta
Dabur Baidyanath Dindayal



Graphs for Physico-chemical parameters of different brands of Ashwagandharishta





S. No.	Properties	Dabur	Baidyanath	Dindayal
1.	pH	C	C	C
2.	Specific gravity at 25°C	C	C	C
3.	Total solids (% w/v)	NC	NC	C
4.	Total alcohol content (% v/v)	NC	NC	NC
5.	Test for methanol (Esterification test)	C	C	C
6.	Reducing sugar	C	C	C
7.	Non-reducing sugar	C	C	C
8.	Heavy metal concentration (ppm)			
a.	Lead	C	C	C
b.	Cadmium	C	C	C
9.	Microbial contamination			
a.	Total bacterial count	C	C	C
b.	Total fungal count	C	C	C

The results of parameters are as follows

Ashwagandharishta of *Dabur* was in liquid state which was clear and without frothing, of Dark brown color, pleasant odor and Astringent taste. This preparation had pH value of 4.3. The weight per ml and specific gravity values were 1.03 mg/ml and 1.06 respectively. The Total Solid content and Total Alcohol content of the preparation were 12.40% w/v and ≈0% respectively. Viscosity and Relative Surface Tension was found to be 1.255 cp and 46.06 dynes/cm. The preparation showed absence of methanol in the qualitative tests. The total sugar, reducing sugar and non-reducing sugar values were found to be 26.15%, 25.48% and 0.67% respectively. The concentrations for heavy metals Lead and Cadmium were found to be 0.156 and 0.010 respectively which were within the prescribed limits. The sample also passed the test for microbial contamination. Phytochemical screening revealed the presence of Glycosides,

Carbohydrates, Reducing sugars, Amino acids, Steroids, Tannins and Terpenoids.

Ashwagandharishta of *Baidyanath* was in liquid state which was clear and without frothing, of Light brown color, pleasant odor and Astringent taste. This preparation had pH value of 3.6. The weight per ml and specific gravity values were 1.07 mg/ml and 1.10 respectively. The Total Solid content and Total Alcohol content of the preparation were 17.90% w/v and ≈0% respectively. Viscosity and Relative Surface Tension was found to be 1.310 cp and 58.65 dynes/cm. The preparation showed absence of methanol in the qualitative tests. The total sugar, reducing sugar and non-reducing sugar values were found to be 20.61%, 20.03% and 0.58% respectively. The concentrations for heavy metals Lead and Cadmium were found to be 0.180 and 0.024 respectively which were within the prescribed limits. The sample also passed the test for microbial

contamination. Phytochemical screening revealed the presence of Glycosides, Carbohydrates, Reducing sugars, Amino acids, Steroids, Tannins and Terpenoids.

Ashwagandharishta of *Dindayal* was in liquid state which was clear and without frothing, of Light brown color, pleasant odor and Astringent taste. This preparation had pH value of 3.9. The weight per ml and specific gravity values were 1.105 mg/ml and 1.149 respectively. The Total Solid content and Total Alcohol content of the preparation were 26.60% w/v and $\approx 0\%$ respectively. Viscosity and Relative Surface Tension was found to be 1.987 cp and 56.901 dynes/cm. The preparation showed absence of methanol in the qualitative tests. The total sugar, reducing sugar and non-reducing sugar values were found to be 22.97%, 22.32% and 0.65% respectively. The concentrations for heavy metals Lead and Cadmium were found to be 0.179 and 0.018 respectively which were within the prescribed limits. The sample also passed the test for microbial contamination. Phytochemical screening revealed the presence of Glycosides, Carbohydrates, Reducing sugars, Amino acids, Steroids, Tannins and Terpenoids.

CONCLUSION

Thus, all the parameters of three brands of Ashwagandharishta had approximately similar values and were compatible with the standard values mentioned in the Pharmacopoeias except the preparation of Baidyanath was of light brown color instead of dark brown color, the Total Solid content values of Dabur and Baidyanath were lesser than that of the standard values and none of them showed the presence of any Total Alcohol content within the preparation. There was also a considerable difference among the values of pharmaceutical parameters which represents the existence of

variation among the formulations. The comparative evaluation of three marketed brands of Ashwagandharishta highlights the critical importance of stringent quality control in Ayurvedic formulations. While all brands met basic organoleptic and physicochemical standards, significant variations in phytochemical contents, were observed. These discrepancies suggest that although the products are generally compliant with regulatory standards, their therapeutic efficacy could vary, impacting consumer outcomes. This study underscores the necessity for more rigorous and standardized quality control protocols to ensure consistency and reliability in herbal products. Such measures would not only enhance the therapeutic efficacy of Ashwagandharishta but also build greater consumer trust in Ayurvedic medicine. Therefore, continuous monitoring and benchmarking of these products are essential for maintaining high-quality standards in the Ayurvedic industry.

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Conflict of interest

We declare that this is a self-funded research work and the author has not received any kind of financial grant from any organization. We have no conflict of interest.

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