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### **Research Article**

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# Standardization of different marketed brands of Ashokarishta: An Ayurvedic formulation

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### Abstract

The use of Ayurveda has been in India since a long period. Many herbal formulations have proved to be effective in the prevention and treatment of many life-threatening diseases. Asavas and Arishtas have been used as medicine for over 3000 years as appetizer and stimulant. In the present study different marketed brands and in-house formulation of Ashokarishta were thoroughly evaluated for their organoleptic characteristics and physicochemical parameters, to establish a routine procedure for standardization of these Ayurvedic formulations. The organoleptic tests performed include colour, odour and taste whereas the physicochemical parameters evaluated were alcohol content, water and alcohol soluble extractive, pH, total solid content, density surface tension and viscosity. In present communication, a TLC method was developed for the evaluation of Ashokarishta by quantitative estimation of major compound kaempferol. Moreover, phytochemical screening was performed for the detection of carbohydrates, proteins, alkaloids, tannins, glycosides, steroids, flavonoids and amino acids.

**Keywords:** Ashokarishta, Organoleptic parameters, Physicochemical parameters, Kaempferol.

### Introduction

Ayurveda is a traditional Indian medicinal system being practiced for thousands of years. Asava and Arishta are unique dosage form discovered by Ayurveda having indefinite shelf life and it was said that the "older the better it is". Because this dosage form has an inherent attribute of continuous hydro-alcoholic extraction and probably formation of natural analogues of the chemical compounds present in the medicinal plants.<sup>1</sup> Ayurveda contains 8 branches of sciences and 10 different diagnostic tools based on *tridosha* theory (three humors of body). Ayurveda also advocates a system of prevention of diseases stipulating a set of practices as daily routine (*Dinacharya*) and seasonal routine (*Ritucharya*). They are herbal teas, infusions, decoctions, tinctures, capsules and powders, infused oils, ointments, creams, lotions etc. along with *arishtas* (fermented decoctions) and *asavas* (fermented infusions).<sup>2</sup>

Ashokarishta is an Ayurvedic herbal remedy with autogenous fermented alcohol with Ashoka as the chief ingredient. It is one of the most popular remedy for 'female disorders' or for menstrual disorder and female hormonal imbalances. Nourishes the blood & the reproductive system, maintains healthy production of female hormones, helping to regulate the menstrual cycle & ease the transition into menopause.<sup>3</sup> It sounds useful for PMS (premenstrual syndrome) and menstrual discomforts. Ashokarishta also relieves back-ache and abdominal pain.

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The tree is regarded as a guardian of female chastity.<sup>4</sup>

The object of this study was to determine the level of alcohol content, water and alcohol soluble extractive, pH, total solid content, density, surface tension and viscosity in different brands of Ashokarishta to establish a routine procedure for preparation.

### Materials and methods

### Method of Preparations of Ashokarishta

In preparation of Ashokarishta, Each 50ml contain, Ashoka (11.16gm), Water (114.27ml), Guda (22.32gm), Dhataki (1.785gm), Shweta Jira (0.112gm), Musta (0.112gm).<sup>5</sup>

The basic were first cleaned and rinsed in water to get rid of dirt. For preparation of arishta a decoction was obtained by boiling the drugs in the specified volume of water used should be cleaned, cleared and potable. When the extracts are obtained the sugar (cane sugar), jaggery and or honey are added and completely dissolved. Sometimes any one or more of these sugary substances are omitted if so directed in the recipe. The sugar jaggery and Honey should be pure the jaggery to be added should be very old (prapurana) because fresh jaggery aggravates kapha and suppresses the power of digestion. The flavoring agents are coarsely powdered and added to sweetened extract. The earthen pot or jar intended for fermenting the medicine is tested for weak spot and cracks and similarly lid is chosen.<sup>6</sup>

### **Collection of Ashokarishta**

Three different brands Ashokarishta were purchased from local market and in-house formulation prepared in laboratory scale. The details are shown in table-1.

### Table 1: Brands of Ashokarishta purchased from market

Brand Name	Main Ingredients	Uses		
Dabur ashokarishta	Ashoka, Guda, Dhataki, Amalaki, Bibhitaka, Haritaki, Shunthi, Amrasthi. Vasa, Shveta chandana, Daruharidra, Musta, Utpala, Shveta jiraka and Water.	Relieves backache and abdominal pain, reduces irritation, improves strength and stamina, ensure active and energetic life throughout the month.		
Sandu ashokarishta	Ashoka, Guda, Dhataki, Ajaji, Mustaka, Bibhitaka, Amalaki, Amrasthi, Jiraka, Vasa and Condana.	Raktapradar and shwetapradar.		
Baidhyanath ashokarishta	Ashoka, Guda, Dhataki, Ajaji, Musta, Sunthi, Daruharidra, Utpala, Haritaki, Bibhitaka, Amalaki, Amla, Sveta jiraka, Vasa, Condana.	Excellent tonic for women, relieves backache, stomachache, headache, debility etc.		
In-house formulation	Ashoka, Dhataki, Daruharidra, Jiraka, Sunthi, Condana, Amla, Guda.	Menorrhagia, irregular menses, leucorrhoea, uterine tonia.		

# Evaluation of different marketed brands and in-house formulation of Ashokarishta

### **Organoleptic properties**

All the different brands and in-house formulation of Ashokarishta were evaluated organoleptically for their colour, odour and taste. The results are shown in table-2.

Table 2: Organoleptic properties of Ashokarisht	Table 2:	Organoleptic	properties of	of Ashokarishta
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Formulations	Appearance	Colour	Taste	Odour
Dabur	Liquid	Brown	Sour	Pleasant
Ashokarishta				
Sandu	Liquid	Brown	Sour	Pleasant
Ashokarishta				
Baidhyanath	Liquid	Dark brown	Sweet	Pleasant
ashokarishta				
In-house formulation	Liquid	Dark brown	Sour	Pleasant

### **Physicochemical properties**

All the three different marketed formulations and in-house formulation of Ashokarishta were evaluated by determining its physicochemical parameters. Physicochemical parameters include alcohol content, water and alcohol soluble extractive, pH, total solid content, density, surface tension and viscosity.

# Determination of alcohol content, water and alcohol soluble extractive

Values of Alcohol content, water soluble extractive and alcohol soluble extractive were determined as per the method described in Indian Pharmacopoeia<sup>7</sup> and the results are shown in table-3.

### **Determination of pH**

The digital pH meter was used for the pH measurement after calibration with buffer solution. pH was noted for all brands of Ashokarishta after opening the bottle and 7 days and 14 days after opening the bottle.<sup>8</sup> The results have been shown in the table-3.

### Table 3: Physicochemical properties of Ashokarishta

### Determination of total solid content

The total solid content was calculated for each brand of Ashokarishta. 10ml of each brand of Ashokarishta were taken in pre-weighed petri dish and dried under oven. The total solid content was calculated in % w/v basis.<sup>9</sup> The results are shown in table-3.

### **Determination of density**

Density of all the samples was determined by using pycknometer10 and the results for different formulations are shown in table-3.

### **Determination of surface tension**

Surface tension provides the information regarding the structure of molecule. Surface tensions of all the samples were determined by using Stalagnometer<sup>10</sup> and the results have been shown in table-3.

Physicochemical	Formulations					
Parameters	Dabur	Sandu	Baidhyanath	In-house		
	ashokarishta	ashokarishta	ashokarishta	formulation		
pH	$3.93\pm0.91$	$3.72 \pm 1.21$	$4.13\pm0.52$	$3.92 \pm 0.42$		
Alcohol content (%v/v)	5.1	6.8	8.7	5.3		
Water-soluble extractive (%w/w)	10.1	11.5	10.2	11.2		
Alcohol-soluble extractive (%w/w)	10.7	9.3	9.1	10.4		
Density (gm/cm <sup>3</sup> )	1.042	1.05	1.068	1.08		
Surface tension (dynes/cm)	60.555	80.484	60.485	72.4		
Viscosity (cps)	2.335	1.067	3.531	2.56		
Total solid content (%w/v)	15.39	15.40	21.76	20.3		

### **Determination of viscosity**

Viscosity for all the samples was determined by using Ostwald viscometer <sup>11</sup> and results are shown in table-3.

### **Phytochemical screening**

 Table 4: Phytochemical screening

Active phytoconstituents like carbohydrates, alkaloids, glycosides, tannins and flavonoids were identified in all three marketed formulations and in-house formulation of Ashokarishta<sup>12</sup> as shown in table-4.

	Formulations						
Phytoconstituents	Dabur	Sandu	Baidhyanath	In-house			
	ashokarishta	ashokarishta	ashokarishta	formulation			
Carbohydrates	+	+	+	+			
Proteins	_	_	_	_			
Alkaloids	+	+	+	+			
Glycosides	+	+	+	+			
Steroids	-	_	_	_			
Tannins	+	+	+	+			
Amino acids	_	_	_	_			
Flavonoids	+	+	+	+			
		+ indicat	tes presence: – indicates	s absence			

## Identification of marker constituents of different marketed and in-house formulations by thin layer chromatography

**Test Solution:** To 50ml of sample add 50ml of distilled water and partitioned in separating funnel using n-hexane then after again partitioned with chloroform and then with ethyl acetate. After partitioning, sample was dried on a water bath at a definite temp. To 20mg of powdered sample, sufficient quantity of methanol was added for spotting.

**Solvent System:** Toluene: Ethyl Acetate: Acetic Acid (5: 4: 1).

**Procedure:** Applied test solution on activated Silica gel G plate of uniform thickness of 0.5mm. The plates were developed in the solvent system.

**Visualization:** The plates were examined under ultraviolet light at 366nm and visualized as fluorescent green in color as in fig.1

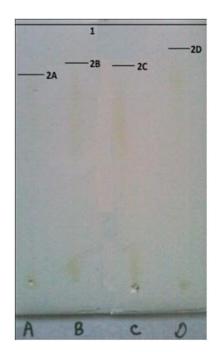


Figure 1: TLC profile of test solution of all three marketed formulations and in-house formulation. 1- Distance traveled by solvent, 2 - Distance traveled by solute. A- Dabur ashokarishta, B- Sandu ashokarishta, C- Baidhyanath ashokarishta, D- Inhouse formulation

**Evaluation:** The Rf value of standard Kaempferol (0.64) was found almost equal with the Rf of all the three marketed products and in-house formulation as shown in table-5.<sup>13</sup>

Table 5:	TLC	Screening	of	Ashokarishta
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Solvent system used	Detection	Development of	<b>Rf</b> value			
		colour	Α	В	С	D
Toluene: Ethyl Acetate:	UV-light	Fluorescent Green	0.62	0.61	0.637	0.655
acetic acid	(366nm)					
A – Dabur ashokarishta, B – Sandu ashokarishta, C – Baidhyanath ashokarishta, D – In-house formulation						

### Results

All the three marketed brands and in-house formulation of Ashokarishta were organoleptic and physicochemical tested and results are reported in table-2 and table-3 respectively. Maximum total alcohol content was found in Baidhyanath ashokarishta whereas minimum total alcohol content in Dabur ashokarishta. pH for different formulations were 3.93 (Dabur ashokarishta), 3.72 (Sandu ashokarishta), 4.13 (Baidhyanath ashokarishta) and 3.92 (in-house formulation). Water- and alcohol-soluble extractive value in each of the marketed brands and inhouse formulation of Ashokarishta was found to be different. Maximum total solid content was observed in Baidhyanath ashokarishta (21.76) whereas minimum total solid content was observed in Dabur ashokarishta (15.39). Maximum surface tension was found in Sandu ashokarishta (80.484) and minimum in Baidhyanath ashokarishta (60.485). Maximum viscosity was found in Baidhyanath ashokarishta (3.531) and minimum in Sandu ashokarishta (1.067). The result obtained for the various chemical tests reveals that the phytoconstituents like carbohydrates, alkaloids, glycosides, tannins and flavonoids were present in each of marketed formulations and in-house formulation as shown in table-4. TLC estimation of different brands and in-house formulation of Ashokarishta shows the presence of Kaempferol in all the marketed and in-house formulations with the Rf value as shown in table-5.

### Discussion

In traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extracts, fresh plant parts, juices and crude powder. Therefore, medicinal plant parts should be authentic and free from microbial contamination. This is the reason why the World Health Organization has set specific guidelines for the assessment of safety, efficacy and quality of the herbal medicines as a prerequisite for global harmonization.<sup>14</sup> Still, very few Ayurvedic industries follow Good Manufacturing practices (GMPs) and are ISO-certified.

This study was done with the aim to understand the benefits of Ayurvedic formulations like arishtas and need to standardize them. In the present investigation, we have evaluated the different brands of Ayurvedic marketed preparations and in-house formulation of Ashokarishta. The present finding have shown that the various physicochemical parameters like pH, alcohol content, water- and alcohol-soluble extractive value, total solid content, viscosity, surface tension etc. was found to be different in each of the marketed formulation and in-house formulation of Ashokarishta. From this study we have found that the alcohol content of all brands and in-house formulation of Ashokarishta are lower than the acceptable limit of alcohol content. Thin layer chromatographic studies showed the presence of active principles like Kaemferol on Rf value for the sample A (0.62), B (0.61), C (0.637) and D (0.655) which was more close to the standard 0.64 Rf with prominent green colouration.

### Conclusion

The present investigation showed that the alcohol content, water-soluble extractive value, alcohol-soluble extractive value, total solid content, viscosity, surface tension, phytochemical screening etc. was found to be different in leading brands and in-house formulation of Ashokarishta. TLC estimation of all marketed and in-house formulations of Ashokarishta shows the presence of Kaempferol and this is the best method for qualitative evaluation of Ashokarishta in lab scale. Study of such formulations in current scenario is of immense importance because asavas and arishtas, the self-fermented products can undergo continuous chemical transformation which goes on beyond hydro-alcoholic extraction of the suspended material. This may result in novel natural molecules with enhanced therapeutic activity.

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