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Review on Quality Evaluation of Ashokarishta: An Ayurvedic Formulation



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ABSTRACT

Ashokarishta is self-generated alcoholic preparation explained in Classics. It is a multi-indicated formulation used widely in all Gynecological disorders. It consists of 15 drugs, which is considered a boon to women. Quality control (Q.C) parameters helpful in the evaluation of the safety and efficacy of the Ayurvedic Formulations. In this present review article, different methods of analysis explained in published articles and their importance have been highlighted. Different analytical methods explained in published articles for standardization and variation in results of the analytical parameters of Ashokarishta will be discussed. Reviewing of 11 articles acts as evidence for multi-indications and maximum utility of Ashokarishta in the present time. Quality Control parameters aid for assurance of genuinity of raw drugs, method of preparation, and proves therapeutic efficacy of Ashokarishta.



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INTRODUCTION:

Ashokarishta is a polyherbal formulation and described in Bhaishajya Ratnavali¹ and Sahasrayogam². Ashokarishta consists of 15 drugs namely Ashoka (*Saraca indica*), Dhataki (*Woodfordia floribunda*), Ajaji (*Nigella sativa*), Musta (*Cyperus rotundus*), Shunti (*Zingiber officinale*), Daruharidra (*Berberis aristhta*), Utpala (*Nymphae stellata*), Haritaki (*Terminalia chebula*), Bibhitaki (*Terminalia belerica*), Amalaki (*Embelica Officinalis*), Amrasthi (*Mangifera indica*), Jeeraka (*Cuminum cyminum*), Vasa (*Adhatoda vasica*), Chandana (*Santalum album*) and Guda. The prime drug is Ashoka, hence called Ashokarishta. Ashokarishta indicated in Raktapradara (DUB), Pida (Pain), Jwara (Fever), Raktapitta (Bleeding disorder), Arsha (Fles), Agnimandya (loss of appetite), Aruchi (Loss of taste), and Shopha (Swelling). In practice, Ashokarishta proved its efficacy in almost all gynecological disorders.

The Quality Control parameters aid to explore the safety and efficacy of the product in different indications mentioned in the classics. Quality Control Parameters helps researchers for a better understanding of Ayurvedic principles of Formulation preparations. Development in analytical techniques like TLC, HPTLC, are used as Fingerprinting tools for assuring genuinity and efficacy of the formulation. So figuring out these developed techniques bridge the gap between Classical principles and Modern parameters. In this present review article, different methods of analysis published in different articles and their importance have been highlighted.

R.Govindarajan et.al(2008), selected three compounds - gallic acid, protocatechuic acid, Rutin, and their concentration for estimation of Phenols. LC-PDA system used for Standardization of Ashokarishta based on quantification and limit of detection of above compounds. Selection of the mobile phase as with 0.3% phosphoric acid and Stationary phase as methanol and water with peak purity ($\lambda = 200-400$ nm), LC-PDA conducted. The study concluded that based on the Gallic acid, Protocatechuic acid, and Rutin the Standardization of Ashokarishta can be done through LC-PDA analysis³.

Akhilesh. K. Mishra et.al (2012), explored the HPTLC method for Standardization of Ashokarishta. Mobile Phase as Toulene: Ethyl acetate: Acetate acid (5:4:1). Four Marketed samples of Ashokarishta procured, and its Physical, Physico-chemical, and HPTLC values

are listed. The author concluded that based on the values, standardization of market samples of Formulations⁴.

N. Vador et.al (2012), explained the Spectrophotometry method for estimation of the functional groups like Total Phenolics, Total Alkaloids, Total Flavonoids, and Total Saponins. The Standardization of 3 Ashokarishta samples (2 marketed samples and an in-house sample) with 700nm UV/Vis Spectrophotometer for quantification of functional groups. The author concluded that the Spectrophotometric method for the quantification of phytoconstituents is simple, fast, and easy for performing⁵.

Dushing Yogesh A et.al, (2012) the anti-oxidant assay of Ashokarishta was performed through UV-Vis Spectrophotometry. The author procured market samples of Ashokarishta - Baidyanath, Rasashala, Sandu Brothers, Vaidyanath. Estimation of Total Phenols, Total Flavonoids, Free radical Scavenging, Reducing Power Assay, Superoxide anion scavenging activity analyzed and values were collected. The Scavenging activity of free radicals of samples found to be in the following order as Vaidyaratnam, Rasashala, Baidyanath, Sandu Brothers, and Reducing Power Assay activity of samples are Vaidyaratnam, Rasashala, Baidyanath, and Sandu Brothers. The study concluded that Ashokarishta of Vaidyaratnam showed higher anti-oxidant activity among four samples⁶.

Nikhil Kumar Singh et.al (2012), estimation of water-soluble vitamins in 7 different Asava-Arishta preparations through the HPTLC method. Mobile Phase as Water, Methanol, Acetic acid, Pentane Sulphonic acid in the ratio of 73:26.5:0.5 with 1.92 gm estimated values of samples help to prove the action of Ashoskarishta as a general tonic⁷.

Tekeshwar Kumar et.al,(2013) explored the TLC method for standardization of four samples of Ashokarishta [3 Market samples (Dabur, Sandu, Baidyanath) and In-house formulation]. The author carried out Physico-chemical analysis and then performed TLC for Standardization with the marker compound as Kaempferol. The author explained sample preparation for the TLC method as – To 50 ml of sample add 50 ml distilled water and separating should be done through separating funnel using n-hexane, then chloroform and then again with ethyl acetate. Then a dried sample or extract of 20 mg, added with the required quantity of methanol. The TLC was performed using mobile phase as Toulene, Ethyl Acetate, and Acetic acid in the ratio of 5:4:1. The author concluded that Physico-

chemical analysis showed variations but TLC showed the presence of Kaempferol. So Author concluded that TLC aids as a Qualitative Evaluation of Ashokarishta⁸.

Chandas Das et.al,(2017) explained variations of values of Physico-chemical parameters (PH, Water-soluble extract, Alcohol soluble extract, Sugar percentage) of four marketed samples of Ashokarishta. The author opines that variations in results were due to change in the collection of drugs from different places and in different seasons. The analysis assures the safety and efficacy of formulations which depends on from collection to finished packaged product. So the author highlights the importance of standardization of samples for marketing it worldwide⁹.

Mohini Kuchekar et.al,(2017) on Analysis like Preliminary evaluation, Physico-chemical analysis (Alcohol content, pH, Density, Viscosity, Surface tension, Total solid content), and their results were tabulated¹⁰.

Vinothkanna Annadurai et.al,(2018) proved the presence of anti-oxidants in herbal ingredients aid promotes reactions to stabilize free radicals¹¹.

C. DAS et.al, (2019) on different Analytical methods for standardization of Asava- Arishta's collected different market samples of Ashokarishta namely Baidyanath, Dabur, Zandu, and in-house preparation. LC with photodiode array identified gallic acid, protocatechuic acid, and rutin compounds in Ashokarishta samples¹².

Rajesh Kumar Sharma et.al,(2020) Standardization of Ashokarishta of market samples namely Dabur, Zandu, Baidyanatha, and Patanjali. Procured samples were analyzed for organoleptic, Physico-chemical, Phytochemical Screening. The study concluded that variation of parameters observed due to different sources and time of collection of herbs, method of preparation¹³.

RESULTS:

Table 1: List of Results of Preliminary Parameters of Ashokarishta mentioned in Published Articles

Sl.No	Parameters	Standard ¹⁴	Tekeshwar Kumar ⁸ et.al				Mohini Kuchekar ¹⁰ et.al	Rajesh Kumar Sharma ¹³ et.al			
			Dabur	Sandu	Baidyanath	In-house preparation		Dabur	Zandu	Baidyanath	Patanjali
1.	Appearance	Clear without Frothing	Liquid	Liquid	Liquid	Liquid		Liquid	Liquid	Liquid	Liquid
2.	Colour	Dark brown	Brown	Brown	Dark Brown	Dark Brown	Blackish brown	Brown	Brown	Dark Brown	Dark Brown
3.	Odour	-	Pleasant	Pleasant	Pleasant	Pleasant	Aromatic	Pleasant	Pleasant	Pleasant	Pleasant
4.	Taste	Astringent	Sour	Sour	Sweet	Sweet	Sweet and Astringent	Sour	Sour	Sweet	Sour

Table 2: List of Results of Physico-chemical Parameters of Ashokarishta

Sl. No	Parameters	Standard ¹⁴	Akhilesh.K.Mishra ⁴ et.al,				N.Vador ⁵ et.al,			Tekeshwar Kumar ⁸ et.al,				Chandan Das ⁹ et.al				Mohini Kuchekar ¹⁰ et.al,	Rajesh Kumar Sharma ¹³ et.al			
			A.1	A.2	A.3	A.4	F1	F2	F3	D	S	B	I	B	D	Z	I		D	Z	B	P
1.	pH	3.5-4.5	4.3	3.5	3.9	3.8	4.3	4.14	4.63	3.93	3.72	4.13	3.92	4.13	-	-	4.63	4.65	3.93	3.72	4.13	3.92
2.	Specific gravity		1.084	1.278	1.118	1.124	1.054	1.062	1.132	-	-	-	-	-	-	-	1.132	-	1.06	1.09	1.07	0.996
3.	Alcohol Content	5-10%	12.4	3.96	11.99	6.67	11.03	5.5	7.27	5.1	6.8	8.7	5.3	8.7	5.1	-	7.27	10.03	6.24	2.51	2.51	2.51
4.	Total solids	Not less than 11%	12.75	11.96	12.14	4.75	-	-	-	15.39	15.40	21.76	20.3	21.76	15.39	15.40	20.3	18	16	32	25	25
5.	Sugar Percent age	RS- not less than 5.05%	RS- 9.44	RS- 9.15	RS- 9.74	RS- 9.42	20.70	21.302	32.606	-	-	-	-	-	-	-	3.26 %	-	-	-	-	-
6.	Density		-	-	-	-	-	-	-	1.042	1.05	1.068	1.08	-	-	-	-	1.088	1.032	1.04	1.056	1.042
7.	Viscosity		-	-	-	-	-	-	-	2.335	1.067	3.531	2.56	3.531	2.335	1.067	2.56	3.4852	2.344	1.056	3.531	2.46
8.	Refractive index		1.365	1.383	1.374	1.381	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.	Surface tension		-	-	-	-	-	-	-	60.555	80.484	60.485	72.4	60.485	60.555	80.484	72.4	97.2778	60.566	78.488	60.456	70.544

Note: *Ashokarishta samples of different market samples and In-House preparation. A.1 indicates Ashokarishta sample 1, A.2- Ashokarishta sample 2, A.3- Ashokarishta sample3, A.4- Ashokarishta sample4, F1- Ashokarishta sample 1,F2- Ashokarishta sample2, F3- Ashokarishta sample3, D- Dabur, S- Sandu, B- Baidyanath, I-In-house Ashokarushta formulation, B- Baidyanath, D-Dabur, Z-Zandu, I- In-house Ashokarushta formulation, D- Dabur, Z-Zandu, B- Baidyanath, P-Patanjali.

Table 3: List of Results of Phytochemical Analysis

Sl. No	Parameters	Tekeshwar Kumar ⁸ et.al				Chandan Das ⁹ et.al				Rajesh Kumar Sharma ¹³ et.al,			
		D	S	B	I	B	D	Z	I	D	Z	B	P
1.	Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
2.	Proteins	-	-	-	-	ND	ND	ND	ND	-	-	-	-
3.	Alkaloides	+	+	+	+	+	+	+	+	+	+	+	+
4.	Glycosides	+	+	+	+	+	+	+	+	+	+	+	+
5.	Tannins	+	+	+	+	+	+	+	+	+	+	+	+
6.	Aminoacids	-	-	-	-	ND	ND	ND	ND	-	-	-	-
7.	Steriods	-	-	-	-	ND	ND	ND	ND	-	-	-	-
8.	Flavanoids	+	+	+	+	+	+	+	+	+	+	+	+

Note: *Ashokarishta samples of different market samples and In-House preparation. D indicates Ashokarishta sample of Dabur manufacturing company, S- Sandu, B- Baidyanath, I- In-house Ashokarishta formulation, B- Baidyanath, D-Dabur, Z-Zandu, I- In-house Ashokarishta formulation, D- Dabur, Z-Zandu, B- Baidyanath, P-Patanjali.

*ND indicates not done.

Table 4: List of Values of TLC

Sl.No	Standard AFI ¹⁴ Volume		Tekeshwar Kumar ⁸ et.al, (Kaempferol)				C. Das ¹² et.al, (Kaempferol)		
	Reagents	Values	Reagents	D	S	B	I	Reagents	Samples
1.	Toulene: Ethyl acetate: Acetic acid in 5:4:1	Kaempferol- 0.64 light green	Toulene: Ethyl acetate: Acetic acid in 5:4:1	0.62	0.61	0.637	0.655	n-butanol: galacial acetic acid: water in 4:4:2	In -house sample: Yellowish grey, Grey, Violet UV-365nm- Brown colour fluorescent spot, and turns red after spraying with alcoholic KOH
								Toulene: Ethyl acetate: Acetic acid in 5:4:1	Rf value of four brands close near to standard Kaempferol value

Note: *Ashokarishta samples of different market samples and In-House preparation. D indicates Ashokarishta sample of Dabur manufacturing company, S- Sandu, B- Baidyanath, I- In-house Ashokarishta formulation,

Table 5: List of Values of HPTLC Analysis:

Akhilesh.K.Mishra ⁴ et.al (Gallic acid)					Nikhil Kumar Singh ⁷ et.al, Concentration range ppm			
Parameters	A001	A002	A003	A004	B1	B2	B3	B6
230 nm	0.55	0.53	0.54	0.55	2.5- 7.5	0.5-1.5	175-525	1.0-2.4
257 nm	0.038	0.071	0.1	0.074				

Note: *Ashokarishta samples of different market samples.A001- Ashokarishta sample 1, A002- Ashokarishta sample, A003- Ashokarishta sample 3, B1- Vitamin B1, B2- Vitamin B2, B3-Vitamin B3, and B6- Vitamin B6.

Table 6: List of Values of Quantitative Analysis:

Sl. No	Parameters	R. Govindarajan ³ et.al,	N.Vador ⁵ et.al,			Dushing Yogesh.A ⁶ et.al,			
		Sample (LC-PDA)	F1	F2	F3	B	R	S	V
1.	Total Alkaloids	ND	913	566.67	336.67	ND	ND	ND	ND
2	Total Phenols	ND	20.21	13.75	16.07	9.14	10.56	7.41	13.08
3	Total Flavanoids	ND	236.47	107.67	212.00	5.15	5.33	3.64	10.92
4	Total Saponins	ND	50.38	43.33	36.67	ND	ND	ND	ND
5	Gallic acids	0.04g/ 1000ml	ND	ND	ND	ND	ND	ND	ND
6	Rutin	0.005g/1000ml	ND	ND	ND	ND	ND	ND	ND
7	Protocatechui c Acids	0.004g/1000ml	ND	ND	ND	ND	ND	ND	ND

Note: *Ashokarishta samples of different market samples and In-House preparation. F1 indicates Ashokarishta sample1, F2- Ashokarishta sample 2, F3- Ashokarishta sample 3, B- Baidyanath, R-Rasashala, S-Sandu Brothers, V- Vaidyaratnam

Table 7: List of values of Free radical scavenging assay

Sl.No	Assays		Dushing Yogesh.A ⁶ et.al,				Vinothkanna Annadurai ¹¹ et.al,
	Assays	Parameters	B	R	S	V	
1.	Free radical Scavenging	Free radical Scavenging	7.17	7.89	13.71	5.67	-
		DPPH	ND	ND	ND	ND	408.38
		Hydroxyl radical	ND	ND	ND	ND	40.69
		Hydrogen peroxide	ND	ND	ND	ND	30.79
		Super oxide anion	ND	ND	ND	ND	53.79
		Nitric oxide	ND	ND	ND	ND	50.21
2.	Inhibition of free radical generating H ⁺ - donating ability	ABTS	ND	ND	ND	ND	58.72
		Metal chelating	ND	ND	ND	ND	56.37
		Reducing power	ND	ND	ND	ND	51.08

Note: *Ashokarishta samples of different market samples and In-House preparation. B indicates Ashokarishta sample of Baidyanath manufacturing company, , R-Rasashala, S-Sandu Brothers, V- Vaidyaratnam.

DISCUSSION:

Total of 11 articles published on Analysis of Ashokarista. Among 11 articles, two are on Anti-oxidant activity, seven are on Standardization. Preliminary parameters, Physico-chemical constituents, Phytochemical screening is a commonly used analysis for Standardization.

Organoleptic Characters and Physico-chemical parameters:

Organoleptic and Physico-chemical Analysis depends upon source, time of drug collection, and method of Ashokarishta preparation. Based on the improved analytical parameters, standardization should be done. The sour taste of some samples indicates Shukti Kalpana (Acidic Preparation), Sweet taste indicates excessive addition of Guda. Increased PH (A-4, F3, I9, Sample10) value suggests that kashaya is not prepared appropriately. Alcohol content values are in the range, indicates proper Arishta preparation, therapeutic activity, and stability. Less Value of Total solids than Standard Values indicates that may be that sample filtered before its completion. Total reducing sugar or Reducing sugars values are within a range suggesting the complete fermentation process. Reviewing the above research articles,

we can assume an average range of Density as 1.04-1.08, Viscosity as 1.067-3.531, Refractive index as 1.36-1.383, Surface tension as 60.55-80.484, WSE as 8.17 -13.9%, ASE as 6.85-13.19 %.

Qualitative Assay:

Phytochemicals assay proved the presence of Carbohydrates, Alkaloids, Glycosides, Tannins, and Flavanoids. TLC method is easy and determined the presence of Kaempferol⁸ (marker/active compound). TLC¹² with different Mobile phases is performed which showed different colored spots, but the author not confirmed about functional group or compound which those spots denote. HPTLC Analysis proved the presence of Gallic acid⁴ and water-soluble Vitamins⁷ like B1, B2, B3, and B6.

Quantitative Assay:

UV/Vis Spectrophotometric Analysis quantified Gallic acid (Folin-Ciocalteu reagent), Total Alkaloids (Drangendroff reagent), Total Phenols (Folin-Ciocalteu reagent), Total Saponin (Anisaldehyde reagent), and Total Flavonoids (AlCl₃ reagent). HPTLC method for the quantification of Gallic acid. Presence of Water-soluble Vitamins (B1, B2, B3, B4) and their quantification with UV / Vis Spectrophotometer. Hence, it acts as evidence for the usage of Ashokarishta as a nutrient tonic.

Anti-oxidant Activity Analysis:

The two articles were published on Anti-oxidant activity. The first article proved Anti-oxidant activity with the presence of flavonoids, phenols, free radical scavenging, Superoxide anion scavenging activity using a UV/Vis Spectrophotometer. Second article with DPPH, Hydroxyl radical, Hydrogen peroxide, Superoxide anion, Nitric oxide, ABTS, Metal chelating, Reducing power using UV/ Vis Spectrophotometer. This indicates within four years period, the Anti-oxidant assay improved with the addition of 4 assays.



CONCLUSION:

Ashokarishta is a multi-indicated formulation, mainly practiced in Gynecological disorders. A total of 11 Analytical research articles published was discussed in the present article. Quality control parameters indicate Standards in manufacturing to enhance acceptability

worldwide. The values of these parameters helpful in proving the safety and efficacy of Ayurvedic Products.

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