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Research Article

QUALITY ASSESSMENT OF DIFFERENT MARKETED BRANDS OF ASHOKARISHTA: AN AYURVEDIC FORMULATION

AKHILESH K MISHRA*[1], DR. RANJIT SINGH[2], KAMLESH K MISHRA[3], MANISH K PATHAK[1]

[1]Smt. Vidyawati college of Pharmacy, Jhansi, U.P, India, [2]Shobhit University Meerut, U.P, INDIA, [3] Department of Biochemistry, University of Allahabad, India. Email: akhileshmishra2010@gmail.com.

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ABSTRACT

Ashokarishta is a classical Ayurvedic preparation that is typically used in menorrhagia and in menstrual disorder. Different marketed products of Ashokarishta have different quality, quantity, purity and standard. The present study was aimed at to evaluate the quality standards of various marketed preparation of Ashokarishta which is prepared according to the Ayurvedic Pharmacopoeia of India (API). The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional heritage but also to rationalize the use of natural product in the health care.

Keywords: Ashokarishta, Alcohol content, Extractive value, HPTLC.

INTRODUCTION

Ayurveda translates into *knowledge* (Veda) of *life* (Ayur) and is one of the oldest and still widely practiced medical systems in the Indian subcontinent[1]. The concept of ayurvedic medicine is to promote health, rather than to fight disease, and Ayurveda in daily life aims at maintaining harmony between nature and the "individual" to ensure optimal health[2]. Arishtas and asavas are self-generated herbal fermentations of traditional ayurvedic system[3]. They are moderately alcoholic (up to 12% by volume) and sweetish with slight acidity and agreeable aroma[4]. Presence of alcohol in the preparation shows several advantages, like better keeping quality, enhanced therapeutic properties, improvement in the efficiency of extraction of drug molecules from the herbs and improvement in drug delivery into the human body sites[5].

The quality assessment of herbal formulation is of paramount importance in order to justify their acceptability in modern system of medicines. It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication having purity, safety, potency and efficacy. Thus it becomes the sole responsibility of the manufacture to maintain the quality of Ayurvedic medication[6].

In Asokarishta, the main herb is Asoka (*Saraca asoca* De Wilde) and their main chemical constituent is gallic acid which is responsible for the therapeutic potency of the drug. It is mandatory to analyze the gallic acid concentration in different brands by using High performance thin layer chromatography[7-8].

MATERIAL AND METHOD

Four different brands of Ashokarishta was purchased from the local market of Patiala and stored in refrigerator. All solvents and reagents used were either of analytical or HPLC grade (E. Merck Ltd., Mumbai, India)

Physical and Physicochemical Evaluation

All physical and Physicochemical Evaluation such as alcohol content, pH, Viscosity, refractive index, Total solid content, Alcohol and water soluble extractive value, Specific gravity and percent reducing sugar were determine as per the method prescribed in the Ayurvedic Pharmacopoeia of India.

Apparatus

(a) HPTLC system.—A Linomat-IV automatic sample applicator, TLC Scanner III, WinCATS software, viewing cabinet with dual wavelength UV lamps, Reprostar-3 Vario system, twin trough chambers, immersion device-III, TLC plate heater (all Camag, Muttenz, Switzerland), Hamilton 100 µL syringe (Anchrom Enterprises Pvt Ltd, Mumbai, India).

(b) Pre-coated TLC Plates.—Silica gel $60F_{254}$, 10cm x 10cm, 20cm x 10cm, layer thickness 0.2 mm, with aluminum backing (E. Merck, Darmastadt, Germany).

Chromatographic Conditions

A precise and accurate quantification can be performed in the linear working concentration range of 75 ng/band with good correlation (r^2 = 0.997). The method was validated for recovery, precision, accuracy, robustness, limit of detection (LOD), limit of quantitation (LOQ), and specificity etc. as per ICH guidelines.

Table 1: Summary of the validation parameters for the proposed HPTLC method

Parameters	Value (Gallic acid)
Limit of Detection at 257 nm scanning (LOD)	18.62
Limit of Quantitation at 257 nm scanning(LOQ)	72.39
Accuracy (%)	97.44-99.54
Repeatability (RSD%, n=5) at 75ng/band	0.21
Precision	
Intra-day (RSD%) (n=3)	0.25-0.89
Inter-day (RSD%) (n=3)	0.42-0.89

Samples were applied to silica gel $60F_{254}$ TLC plates (10 × 10 cm) by means of Linomet-IV semiautomatic spotter equipped with 100 µL syringe and operated with a settings of band length 6 mm, distance between the bands was kept 15 mm, distance from the plate edge 15 mm and distance from the bottom of the plate 15 mm with a speed of 8 µL/min. The plates were developed in linear ascending mode for a distance of 8 cm in a vertical twin trough chamber previously saturated for 45 min with the mobile phase Toluene: Ethyl acetate: Acetic acid (5:4:1) under laboratory conditions (temperature 25 ± 3°C and relative humidity 35-40%). The bands on the air-dried plates were scanned with a scanner-III at 230 nm in reflection/absorption mode. The plates were also immersed (dipping time 2 s, dipping speed 5 cm s^{-1}) in freshly prepared vanillin-sulphuric acid derivatizing reagent (vanillin: ethanol: Sulfuric acid - 1g: 95mL: 5mL) followed by heating at 110°C for 10 min. The densitometric digital scanning was performed in the reflectance/absorbance mode, slit width 6.00 mm × 0.40 mm, scanning speed 20 mm s^{-1} and data resolution 10 µm $step^{-1}$. Savitsky-Golay-7 was used for data filtering and the lowest slope for baseline correction in order to integrate the area. For recording of characteristic UV absorption spectra (200–400 nm) of sample track, deuterium lamp was used.

Sample preparation

50 ml of the formulation was dried in vacuum to remove the self generated alcohol then 50 ml water was added in to dissolve the extract and then partitioned successively with 50 ml of n-hexane, chloroform and ethyl acetate. Filtered and concentrated the ethyl acetate extract under vacuum and weighed. 20 mg of residue was dissolved in 1 ml of methanol. Above methanolic solution was applied on a pre-coated Merck Silica gel 60 F_{254} plate and the plate was developed in a suitable solvent system [Toluene: Ethyl acetate: Acetic acid (5:4:1)] in a twin trough chamber up to a length of approximately 8 cm[9].

RESULT AND DISCUSSION

All physical and Physicochemical Evaluation such as alcohol content, pH, Viscosity, refractive index, Total solid content, Alcohol and water soluble extractive value, Specific gravity and percent reducing sugar were determine as per the method prescribed in the Ayurvedic Pharmacopoeia of India.

Samples (A001-A004) were tested for refractive index. All the samples were found to have very close values of refractive indices[10].

Specific gravity of the sample was calculated by Pycnometer and all the calculated data was found to be within the range[11].

Total solid content of Ashokarishta preparation should be more than 11% (API). Results of first three samples were found to be satisfactory but sample (A004) having 4.755% solid content, shows a significant variation from the standard[12].

There is a specified range of alcohol in API (5-11%). The alcohol content of sample A001 was found to be 12.46 % which is more than the specified value and for sample A002 was found to be 3.96 which is less than the specified range. A significant deviation in alcohol content was observed in the samples which could be detrimental to the efficacy of the formulation[13].

Calculated alcohol soluble extractive value and water soluble extractive value of samples was found to be in range of 6.85 – 13.19 % w/w and 8.17-13.91 % w/w respectively. It shows that there are a lot of variations in alcohol and water soluble extractive value which is not a good sign for the therapeutic potential for the formulation[14].

According to API Ashokarishta should not contain less than 5.5% of reducing sugar. All the sample of Ashokarishta were analyzed for the reducing sugar and all the samples were found in accordance to the limits mention in the API[15]. All the physicochemical parameters are summarized in the Table 2.

HPTLC data revealed that sample A003 contains highest amount of gallic acid and sample A001 contains minimum amount of gallic acid at wavelength 257nm. So there was found to be a lot of variations in gallic acid concentration.

Table 2: Physical and physicochemical evaluation of different brand of Ashokarishta.

Parameters	A001	A002	A003	A004
pH	4.3	3.5	3.9	3.8
Refractive Index	1.365	1.383	1.374	1.381
Specific gravity	1.084	1.278	1.118	1.124
Total solid (%w/v)	12.75	11.96	12.14	4.75
Alcohol content (%v/v)	12.46	3.96	11.99	6.67
Alcohol soluble extractive value(%w/v)	7.59	13.19	6.85	9.39
Water soluble extractive value (%w/v)	13.91	8.17	13.84	11.57
% Reducing sugar	9.44	9.15	9.74	9.42

R_f value for standard gallic acid solution was found to be 0.55 and for sample A001, A002, A003 and A004 were found to be 0.53, 0.54, 0.54 and 0.55 respectively. Concentration of gallic acid in sample A001, A002, A003 and A004 at 257 nm was found to be 0.038, 0.071, 0.1 and 0.074 g/100mL respectively.

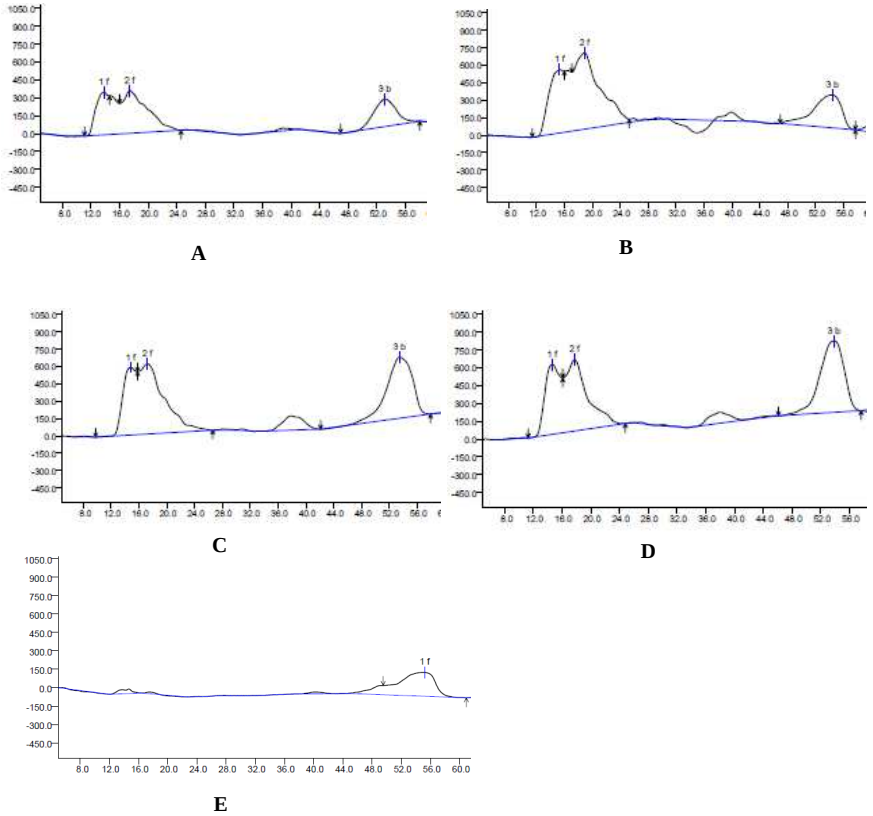

Fig. 1: It shows representative TLC densitogram of Ashokarishta at 257nm. A, B, C and D show HPTLC fingerprinting profile of Samples A0001-A004. E is marker fingerprinting profile of gallic acid.

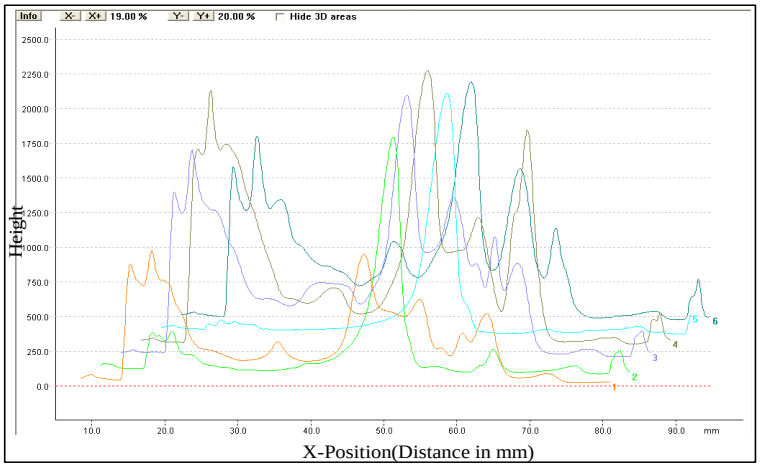

Fig. 2: Three dimensional view of HPTLC profile graph at λ 254 nm

Lane 1,3,4,6 is for sample A001-A004 and lane 2 and 5 for Standard

CONCLUSION

India has a great diversity in medicinal herbal resources. More than 70% of the Indian population uses herbal drugs for the treatment of various diseases, and the manufacture of these medicines is mushrooming. Traditional herbal medicines and their preparations have been widely used in India as well as abroad for many years. But there are only few industrial organizations in India which carry out quality assessment on herbal drugs. So it becomes pertinent to the regulatory authorities to tighten their noose on manufacturers providing substandard products in the market. Stern steps are required to be taken to improve the quality parameters of these formulations. From the study it can be concluded that the recorded levels of alcohol content, total solid content, reducing sugar content, % tannic acid, % water and alcohol soluble extractive value, gallic acid concentration and pH etc in commercially available Ashokarishta could be used to establish and formulate procedures for standardization and quality controlling of these ayurvedic preparations. This is the reason why the World Health Organization and API have set specific guidelines for the assessment of the safety, efficacy, and quality of herbal medicines as a prerequisite for global harmonization it becomes responsibility on part of the manufacturer to market the products of specific quality. Regulatory bodies and the government sector should implement stringent policies to regulate and monitor the manufacture and marketing of herbal formulations.

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