



Phytoconstituents Based Standardization of Two Most Well Known Classical Ayurvedic Arishta Preparations- “*Arjunarishta and Dashamularishta*”

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ABSTRACT

Arishtas are one of the most important traditional Ayurvedic formulations claimed to be beneficial for various ailments. The present investigation evaluated two well known Arishtas available in the market, Arjunarishta [Parthadyarishta] and Dashamularishta with respect to various physico-chemical parameters, active phytochemical groups and microbiological quality parameters. Additionally, a simple and rapid Thin Layer Chromatographic (TLC) fingerprinting was developed for both Arjunarishta and Dashamularishta for evaluation of quality as well as to check batch to batch consistency.

Keywords: Arjunarishta, Dashamularishta, Phytochemical, Thin Layer Chromatography (TLC)

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INTRODUCTION

Now-a-days there is resurgence of interest in Ayurveda in India as well as in the Western countries. This traditional Ayurvedic treatment is aimed at restoring harmony or balance to the mind-body system. Ayurvedic medicines are gaining hold all around the world. Scientific research is increasingly gaining ground in the application of Ayurvedic medicines^{1, 2}. Arishtas are one of the most important Ayurvedic preparations made by soaking the drugs, in the form of decoction, in a solution of sugar or jaggery, as the case may be, for a specified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of the active principles contained in the drugs. The alcohol, so generated, also serves as a preservative. Although these formulations are mentioned in traditional literature and used regularly, their scientific investigation and reporting is essential to strengthen Ayurveda in global market^{2,3}. The present study was designed to assess the quality and consistency of two most well known Arishta preparations, namely Arjunarishta and Dashamularishta. Both the samples were standardized with respect to different physico-chemical parameters, active phytochemical moieties and microbiological parameters. A new simpler and rapid Thin Layer Chromatographic (TLC) profile has been developed for these two products which may ensure the consistency of these products.

MATERIALS AND METHOD

Four different brands of Arjunarishta and Dashamularishta were purchased from local market of Kolkata, West Bengal, India. All the samples were stored in the refrigerator at 4°C-8°C and collected for experiments under aseptic conditions.

Physico-chemical Evaluations

General physico-chemical parameters such as description, pH, total solids, specific gravity, color index, reducing sugar, non-reducing sugar and microbiological parameters such as total microbial plate count, Yeast and mould, *Staphylococcus aureus*, *Salmonella* sp, *Pseudomonas aeruginosa* and *Escherichia coli* were determined as per the method prescribed in the Ayurvedic Pharmacopoeia of India.

Estimation of Total Polyphenol Content⁴

The total polyphenol content was determined by spectrophotometric method, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO). Briefly, 1.0 ml of the diluted sample was transferred in duplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then,

4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The total polyphenol content was expressed with respect to gallic acid. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 20 to 80 µg/ml.

Estimation of Ethanol content

Gas Chromatography was carried out on a Perkin Elmer, Model: Clarus 480; equipped with a capillary column Elite-1 (30 m x 0.25 mm i.d.; part no:-N9316010) and Flame Ionization Detector (FID). Carrier gas used was nitrogen with constant flow rate: 1.8 ml/min. The injector temperature, oven temperature and detector temperatures were set at 120°C, 150°C and 280°C respectively. Analysis was done through Total Chrom software (version 6.3.2).

Development of TLC Fingerprinting Profile

Sample Preparation

10 gm of each sample is taken in 50 ml water and extracted with ethyl acetate (50×3). The combined ethyl acetate layer was filtered and moisture removed after passing through anhydrous sodium sulphate. Ethyl acetate layer was evaporated under vacuum in a rotary evaporator. The residue was reconstituted in 2 ml of methanol and the resulting solution is used as sample solution. 10 & 20 µl of each test solution is applied to the silica gel 60 F₂₅₄ plate as bands of 10 mm. The developing solvent is allowed to raise upto 8 cm.

HPTLC condition

Stationary phase:	TLC aluminium sheet silica gel 60F ₂₅₄ [Merck: 1.05554.0001]
Solvent system:	Toluene: Ethyl acetate: Formic acid: Methanol 30: 30.8:2 [v/v/v/v]
Application:	Linomat 5 automated applicator [CAMAG]
Development distance:	80 mm
Detection:	At 254 nm; 366nm and after derivatisation with Anisaldehyde Sulphuric Acid Reagent

The track details are as follows:

Track 1, 2: Brand 1; Track 3, 4: Brand 2; Track 5, 6: Brand 3; Track 7, 8: Brand 4

RESULTS AND DISCUSSIONS

Organoleptic Evaluations

Organoleptic characteristics of different brands of Arjunarishta samples revealed that these are palatable to use because of astringent taste (Brand 1&2) combined with fine aroma which masks

unpleasant taste and odor of added herbal ingredients. On the other hand two other brands (Brand 3&4) having sour taste does not comply with the organoleptic characteristics as per API. The results are incorporated in Table 1. Organoleptic characteristics of different brands of Dashamularishta samples revealed that these are complying with the organoleptic characteristics as per API. The results are incorporated in Table 2.

Table 1: Organoleptic characteristics of Arjunarishta

Parameter	Specification as per API	Brand Codes			
		Brand 1	Brand 2	Brand 3	Brand 4
Colour	Brown	Brown	Brown	Brown	Brown
Odour	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
Taste	Astringent	Astringent	Astringent	Astringent and sour	Astringent and sour
Appearance	Significant sedimentation	Significant sedimentation	Significant sedimentation	Significant sedimentation	Significant sedimentation

Table 2: Organoleptic characteristics of Dashamularishta

Parameter	Specification as per API	Brand Codes			
		Brand 1	Brand 2	Brand 3	Brand 4
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Odour	Alcoholic	Alcoholic	Alcoholic	Alcoholic	Alcoholic
Taste	Bitter	Bitter	Bitter	Bitter	Bitter
Appearance	Significant sedimentation	Significant sedimentation	Significant sedimentation	Significant sedimentation	Significant sedimentation

Physico-chemical Evaluations

Physico-chemical parameters such as pH, total solids, specific gravity, total sugar, alcohol content, absence of methanol and total phenolic content of different brands of Arjunarishta samples were determined. Total sugar, non-reducing sugar content and alcohol content of each brand of Arjunarishta sample were found to be different in comparison to specification as per API. The maximum sugar content (19.10%) was found in Brand-1 and minimum (11.81%) was found in Brand-3. Similarly maximum alcohol content (8.47%) was found in Brand-2 and minimum (2.80%) was found in Brand-3 whereas in Brand-4 the alcohol content is also on the lower side (3.69%) suggesting the improper fermentation. The results are incorporated in Table 3. Physicochemical parameters such as pH, total solids, specific gravity, total sugar, alcohol content, absence of methanol and total phenolic content of different brands of Dasmoolarishta samples were determined. pH, reducing sugar and alcohol content of each brand of Dasmoolarishta sample was found to be different in comparison to specification as per Ayurvedic Pharmacopoeia of India (API). The maximum sugar content (27.60%) was found in Brand-1 and minimum (21.29%) was found in Brand-4. Similarly maximum alcohol content

(7.33%) was found in Brand-1 and minimum (1.52%) was found in Brand-3 whereas in Brand-4 the alcohol content is also on the lower side (4.04%) suggesting the improper fermentation. The results are incorporated in Table 4.

Microbiological Evaluations

The total microbial plate count, yeast and mould counts of all marketed brands of Arjunarishta samples are well with the specified limit as per Ayurvedic Pharmacopoeia of India. All the preparations were found to be negative for the presence of pathogens. The results are incorporated in Table 5. The total microbial plate count, yeast and mould counts of all marketed brands of Dashamularishta samples are well with the specified limit as per Ayurvedic Pharmacopoeia of India. All the preparations were found to be negative for the presence of pathogens. The results are incorporated in Table 6.

Table 3: Physico-chemical properties of Arjunarishta

Parameter	Specification as per API	Observed value of different brands			
		Brand 1	Brand 2	Brand 3	Brand 4
pH	4.0-4.6	4.17±0.06	4.50±0.05	4.35±0.08	4.03±0.03
Total solids (% w/v)	Not Less Than 10.0	27.95±0.24	14.28±0.20	24.17±0.23	14.52±0.17
Specific gravity at 25°C (g/cc)	1.02-1.05	1.10±0.02	1.05±0.03	1.10±0.03	1.05±0.02
Reducing sugar (% w/v)	Not Less than 5.5	19.10±0.21	13.03±0.18	11.81±0.16	17.22±0.21
Non-reducing sugar	Not More Than 0.30	1.12±0.10	1.04±0.17	1.23±0.06	1.10±0.08
Alcohol content (% v/v)	6-12	8.16±0.14	8.47±0.16	2.80±0.22	3.69±0.15
Methanol	Should be Absent	Absent	Absent	Absent	Absent
Total Phenolic content as Gallic acid (% w/v)	-	0.58±0.03	0.54±0.03	0.61±0.02	0.44±0.02

Table 4: Physico-chemical properties of Dashamularishta

Parameter	Specification as per API	Observed value of different brands			
		Brand 1	Brand 2	Brand 3	Brand 4
pH	3.60-3.70	4.28±0.08	4.41±0.07	4.01±0.07	4.02±0.05
Total solids (% w/v)	24-54	27.79±0.22	30.03±0.14	27.61±0.13	22.51±0.27
Specific gravity at 25°C (g/cc)	1.09-1.10	1.10±0.02	1.09±0.04	1.10±0.05	1.09±0.03
Reducing sugar (% w/v)	14-24	27.60±0.24	26.39±0.28	25.77±0.36	21.29±0.41
Non-reducing sugar (% w/v)	Not More than 1.0	0.51±0.03	0.61±0.02	0.49±0.02	0.60±0.04
Alcohol content (% v/v)	5-7	7.33±0.12	9.11±0.38	1.52±0.20	4.04±0.22
Methanol	Should be absent	Absent	Absent	Absent	Absent
Total Phenolic content as Gallic acid (% w/v)	-	0.93±0.04	1.19±0.03	0.94±0.04	1.05±0.02

Table 5: Microbiological evaluation of different marketed brands of Arjunarishta

Parameter	Specification as per API	Brand Codes			
		Brand 1	Brand 2	Brand 3	Brand 4
Total microbial plate count (CFU/g)	Not more than 10 ⁵	<10	<10	15	05

Yeast and Mould (CFU/g)	Not more than 10 ³	<10	<10	<10	<10
Escherichia coli	Should be absent	Absent	Absent	Absent	Absent
Salmonella spp	Should be absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Should be absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Should be absent	Absent	Absent	Absent	Absent

Table 6: Microbiological evaluation of different marketed brands of Dashamularishta

Parameter	Specification as per API	Brand Codes			
		Brand 1	Brand 2	Brand 3	Brand 4
Total microbial plate count (CFU/g)	Not more than 10 ⁵	<10	<10	20	10
Yeast and Mould (CFU/g)	Not more than 10 ³	<10	<10	<10	<10
Escherichia coli	Should be absent	Absent	Absent	Absent	Absent
Salmonella spp	Should be absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Should be absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Should be absent	Absent	Absent	Absent	Absent

Development of TLC Fingerprint Profile

Fingerprinting is now globally accepted as a quality evaluation model of herbal medicine. So, a simple and unique TLC fingerprinting profile has been developed for all marketed brands of Arjunarishta and Dashamularishta samples which could be an effective tool for the evaluation of quality as well as batch to batch consistency. Arjunarishta samples received from Brand 1 to Brand 3 (Track No 1-6) showed similar spots (R_f 0.12, 0.30, 0.58, 0.62, 0.70, 0.72, 0.80) with similar density at 254 nm. However, sample received from Brand 4 showed much lighter spots at R_f 0.12 and 0.30 (Track No 7-8) at same concentration at 254 nm suggesting lesser amounts of active ingredients in the sample in comparison to other marketed samples. The profile is shown in Figure 1. Arjunarishta samples received from Brand 2 to Brand 4 (Track No 3-8) showed similar spots (R_f 0.10, 0.14, 0.32, 0.43, 0.50, 0.60, 0.66, 0.82) with similar density at 366 nm. However, sample received from Brand 1 showed much lighter spots (Track No 1-2) at same concentration at 366 nm suggesting lesser amounts of fluorescence nature ingredients in the sample in comparison to other marketed samples. The profile is shown in Figure 2. Arjunarishta samples received from Brand 2 to Brand 4 (Track No 3-8) showed similar spots (R_f 0.07, 0.30, 0.50, 0.58, 0.70, 0.81) with similar density after derivatisation with Anisaldehyde Sulphuric Acid Reagent. However, sample received from Brand 1 showed much lighter spots (Track No 1-2) at same concentration after derivatisation with Anisaldehyde Sulphuric Acid Reagent suggesting lesser amounts of ingredients in the sample in comparison to other marketed samples. The profile is shown in Figure 3. Dashamularishta samples received from Brand 1 and Brand 2 (Track No 1-4) showed similar spots (R_f 0.05, 0.09, 0.40, 0.44, 0.58, 0.62, 0.64, 0.67, 0.71) with

similar density at 254nm. However, Dashamularishta samples received from Brand 3 and Brand 4 (Track No 5-8) showed lesser no of spots but with higher density at (R_f 0.48, 0.62). The profile is shown in Figure 4. Dashamularishta samples received from Brand 1 to Brand 4 (Track No 1-8) showed similar spots (R_f 0.05, 0.09, 0.16, 0.26, 0.34, 0.42, 0.53, 0.58, 0.67, 0.71, 0.79) with similar density at 366nm. The profile is shown in Figure 5. Dashamularishta samples received from Brand 1 and Brand 2 (Track No 1-4) showed similar spots (R_f 0.05, 0.09, 0.40, 0.44, 0.58, 0.62, 0.64, 0.67, 0.71) with similar density after derivatisation with Anisaldehyde Sulphuric Acid Reagent. However, Dashamularishta samples received from Brand 3 and Brand 4 (Track No 5-8) showed lesser no of spots but with higher density at (R_f 0.48, 0.62). The profile is shown in Figure6. Recently, there has been a shift in Universal trend from synthetic to herbal medicines. Herbal medicines have been known for millennia and highly esteemed for the prevention of disease and ailments. The global market for herbal medicines currently stands at over \$60 billion annually. The sale of herbal medicines is expected to get higher at 6.4% an average annual growth rate. India has been bestowed by Mother Nature with an enormous wealth of medicinal plants and has a great potential to become the leader in the International market. Unfortunately, India's share to international market is not up to the mark due to lack of standardization and quality assessment. Despite of specific guidelines of World Health Organization (WHO) for the assessment of the safety, efficacy and quality of herbal medicines, very few Ayurvedic industries follows Good Manufacturing Practices (GMP) and are ISO certified². In the present investigation, we have evaluated four different brands of Arjunarishta and Dashamularishta available in the market with respect to various physico-chemical and microbiological quality parameters. Additionally, a simple and unique Thin Layer Chromatographic (TLC) fingerprinting profile has been developed for all brands of Arjunarishta and Dashamularishta for evaluation of quality as well as batch to batch consistency. The preparations were purchased from local market of Kolkata, west Bengal, India. All the samples were stored in the refrigerator at 4°C to 8°C and collected for experiments under aseptic conditions. The present findings have shown that organoleptic and alcohol content varies between different brands, although microbiological parameters are acceptable as per Ayurvedic Pharmacopoeia of India.

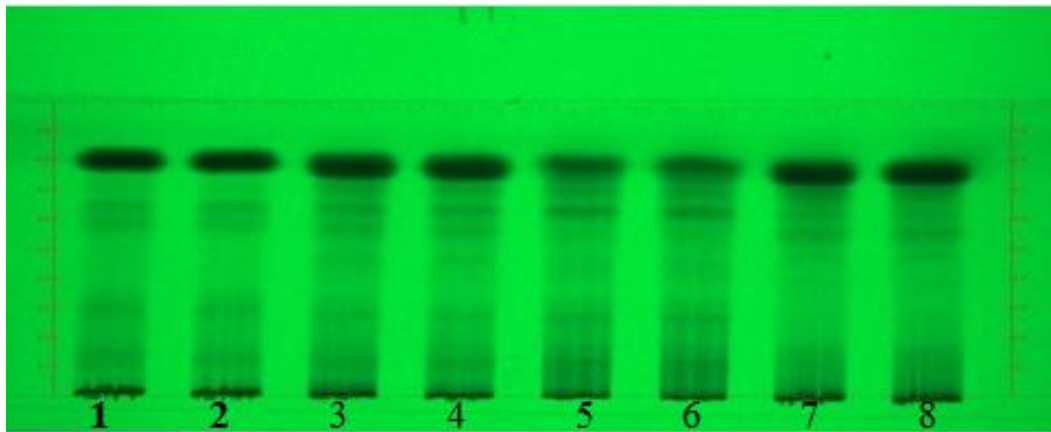


Figure-1: TLC Fingerprinting of Arjunarishta samples at 254 nm

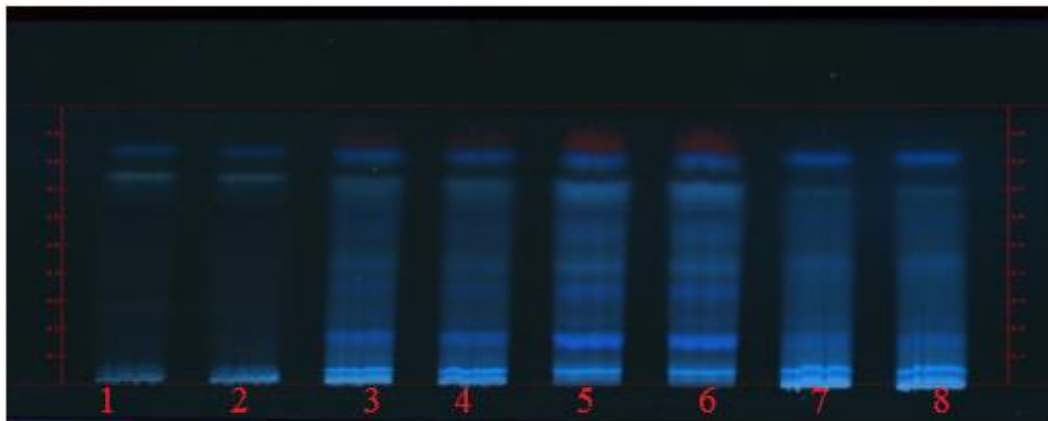


Figure-2: TLC Fingerprinting of Arjunarishta samples at 366 nm

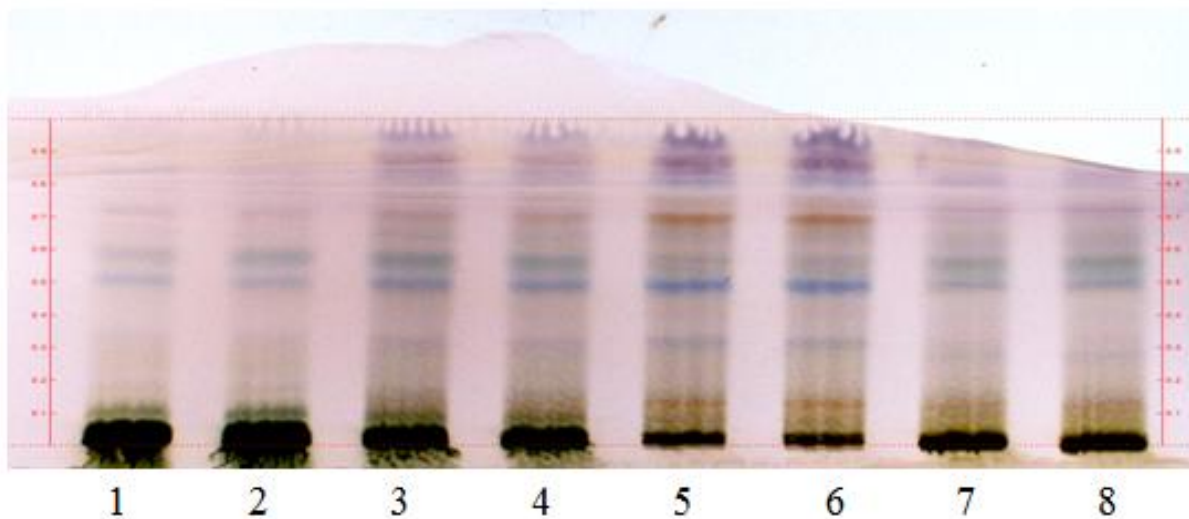


Figure-3: TLC Fingerprinting of Arjunarishta samples after derivatisation with Anisaldehyde Sulphuric Acid Reagent.

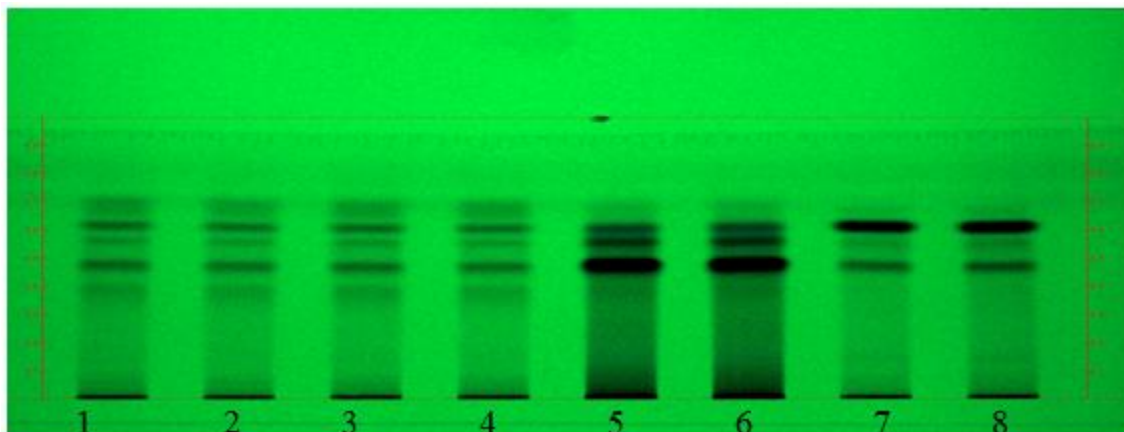


Figure-4: TLC Fingerprinting of Dashamularishta samples at 254 nm

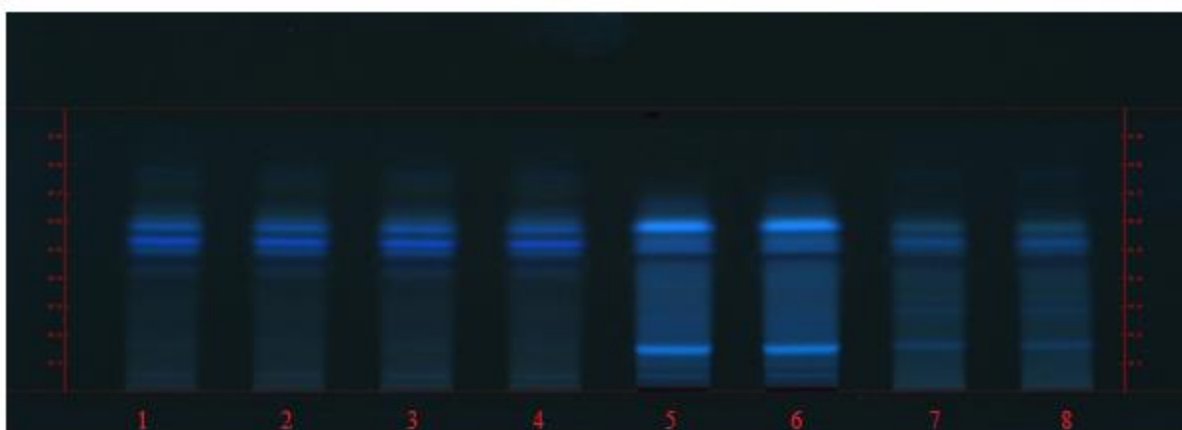


Figure-5: TLC Fingerprinting of Dashamularishta samples at 366 nm

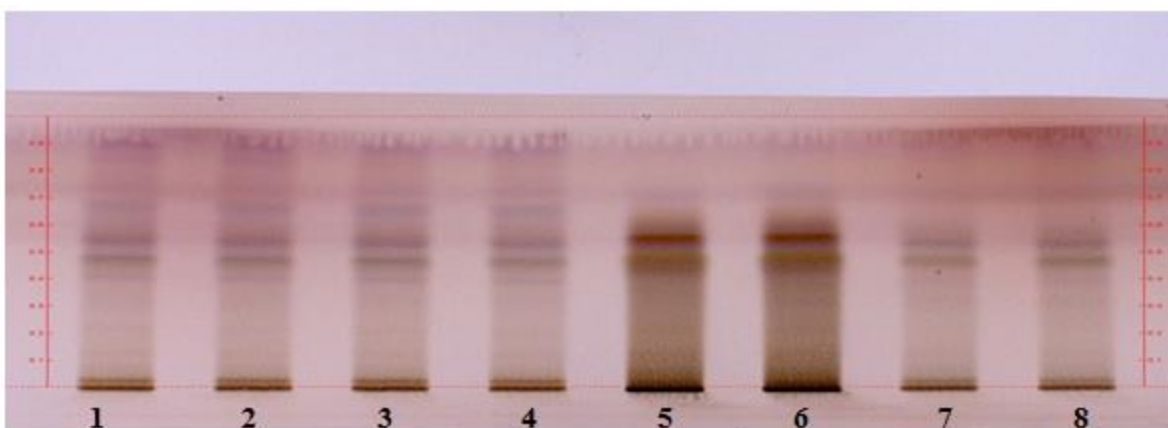


Figure-6: TLC Fingerprinting of Dashamularishta samples after derivatisation with Anisaldehyde Sulphuric Acid Reagent.

CONCLUSION

The present investigation found lack of consistency between different brands of Arjunarishta and Dashamularishta samples which might have a profound bearing on the efficacy of the products although all were found to be acceptable with respect to microbial load. In case of alcohol

content, it was found that the different brands showed varying results which to be regulated and monitored stringently for manufacture and marketing of herbal formulations. Additionally, a simple and rapid TLC fingerprinting profile was developed to ensure the consistency of the products.

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