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Original Research Article

QUALITY ASSESSMENT AND DEVELOPMENT OF TLC FINGERPRINTING PROFILE OF DIFFERENT MARKETED BRANDS OF TWO AYURVEDIC FORMULATIONS, *DRAKSHASAVA* AND *KUMARYASAVA*

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ABSTRACT:
Drakshasava and *Kumaryasava* are two most important traditional Ayurvedic formulations claimed to be beneficial for various ailments. The present investigation evaluated four different brands of *Drakshasava* and *Kumaryasava* available in the market with respect to various physico-chemical and microbiological quality parameters. Additionally, a simple and rapid Thin Layer Chromatographic (TLC) fingerprinting was developed for all brands of *Drakshasava* and *Kumaryasava* for evaluation of quality as well as batch to batch consistency. All the preparations showed acceptable limits of microbial loads but some showed unacceptable levels of alcohol content as per limit given in Ayurvedic Pharmacopoeia of India (5-10%).
Keywords: *Drakshasava*, *Kumaryasava*, Thin Layer Chromatography (TLC), Alcohol

INTRODUCTION:

Asavas are medicinal preparations made by soaking the drugs, in coarse powder form, 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 [1].

Drakshasava is a very important *asava* formulation used to improve digestion, as blood purifier, in the treatment of anaemia, heart disease, abdominal disorder, wound and fever and advised as a choice of remedy in respiratory problems. The chief ingredient of *Drakshasava* is draksha, dried fruits of *Vitis vinifera* [1, 2].

Kumaryasava is another important *asava* formulation used in the treatment of abdominal lump, cough, asthma, piles, epilepsy and neurological disorders. The main ingredient of *Kumaryasava* is Kumari, *Aloe barbadensis* [1, 2].

Now a days there is resurgence of interest in Ayurveda in India as well as in the Western countries. This traditional 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. The World Health Organization (WHO) estimated that nearly 80% of the earth's inhabitants rely on traditional medicines for their primary health-care needs, and most of this therapy involves the use of plant extracts or their active components. This type of treatment prevailed during different times of history in different countries, until about the beginning of 19th century. One of the major drawbacks faced by Ayurvedic

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drug industry is the unavailability of rigid quality control profiles for Ayurvedic medicines. Ayurvedic formulations available in the market are usually not properly standardized and are not assessed for their quality. There is an urgent need for manufacturer, pharmacists and physicians to standardize the products to attain the highest level of safety and efficacy on a consistent basis [2, 3].

The present study was designed to assess the quality and consistency of different marketed brands of two most widely used *asava* preparations with respect to different physico-chemical and microbiological parameters. A new simpler and rapid Thin Layer Chromatographic (TLC) profile has been developed for these two products as the method described in Ayurvedic Pharmacopoeia is lengthy and cumbersome as it involves successive extraction stages.

MATERIALS AND METHODS:

Four different brands of *Drakshasava* and *Kumaryasava* were purchased from local market of Kolkata, west Bengal, India. All the samples were stored in the refrigerator at 8°C and collected for experiments under aseptic conditions.

Physico-chemical Evaluations:

General physico-chemical parameters such as macroscopic description, pH, total solids, specific gravity, 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 Content [4]:

The total polyphenol content was determined by spectrophotometry, 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 Elite-1 column (30 m x 0.25 mm i.d.) and Flame Ionising 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. All the analytical data of GC analysis were based on TotalChrom software (version 6.3.2).

Development of TLC Fingerprinting Profile:

Sample Preparation: 10 gm of each sample is dissolved in 50 ml water and extracted with ethyl acetate (50×3). The ethyl acetate layer was filtered after passing through sodium anhydrous sulphate. Ethyl acetate layer was evaporated to dryness in a rotary evaporator. The residue was reconstituted in 2 ml of methanol and the resulting solution

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is used as sample solution. 10 & 20 µl of each test solution is applied to the silica gel 60 F_{254} plate as bands of 10 mm. The mobile phase is allowed to rise upto 8 cm.

HPTLC condition:

Stationary phase:	TLC aluminium sheet silica gel $60F_{254}$
Mobile phase: [v/v/v/v]	Toluene: Ethyl acetate: Formic acid : Methanol 30: 30.8:2
Application:	Linomat 5 automated applicator [CAMAG]
Development distance:	80 mm
Detection:	At 254 nm**;** 366nm and after derivatisation with Anisaldehyde Sulphuric Acid Reagent [TLC SCANNER 4 by CAMAG]
Evaluation:	win CATS ver. 1.4.6 [CAMAG]

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 *Drakshasava* samples revealed that these are palatable to use because of sweet taste (in case of 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.

Table-1: Organoleptic characteristics of *Drakshasava*

Parameter	**Specification as per API**	**Brand Codes**			
		Brand 1	**Brand 2**	**Brand 3**	**Brand 4**
Colour	Brown	Very dark brown	Dark brown	Very dark brown	Brown
Odour	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
Taste	Sweet	Sour	Sweet	Sour	Sweet
Appearance	Clear	Clear	Clear	Clear	Clear

Organoleptic characteristics of different brands of *Kumaryasava* samples revealed that these are complying with the organoleptic characteristics as per API. The results are incorporated in Table-2.

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Table-2: Organoleptic characteristics of *Kumaryasava*

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	Astringent	Astringent	Astringent	Astringent	Astringent
Appearance	Clear	Clear	Clear	Clear	Clear

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 *Drakshasava* samples were determined. Total sugar content and alcohol content of each brand of *Drakshasava* sample was found to be different. The maximum sugar content (18.50%) was found in Brand-4 and minimum (9.07%) was found in Brand-1. Similarly maximum alcohol content (9.11%) was found in Brand-2 and minimum (1.52%) was found in Brand-1 whereas in Brand-3 the alcohol content is also on the lower side (4.04%) suggesting the improper fermentation. The results are incorporated in Table-3.

Table-3: Physico-chemical properties of *Drakshasava*

Parameter	Specification as per API	Observed value of different brands			
		Brand 1	Brand 2	Brand 3	Brand 4
pH	4.0-4.5	4.56±0.05	4.46±0.06	4.01±0.08	4.05±0.03
Total solids (%w/v)	Not Less Than 25.0	23.96±0.21	25.37±0.24	24.09±0.23	33.10±0.37
Specific gravity at 25°C (g/cc)	1.08-1.20	1.10±0.02	1.10±0.03	1.09±0.03	1.13±0.02
Reducing sugar (% w/v)	Not Less than 16.0	9.07±0.11	13.15±0.18	10.22±0.16	18.5±0.21
Non-reducing sugar	Not More Than 0.80	1.21±0.10	1.69±0.17	1.19±0.06	1.49±0.08
Alcohol content (%v/v)	5-10	1.52±0.10	9.11±0.16	4.04±0.22	7.33±0.12
Methanol	Should be Absent	Absent	Absent	Absent	Absent
Total Phenolic content as Gallic acid (% w/v)	-	0.77±0.03	0.64±0.03	0.54±0.02	0.63±0.02

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Physicochemical parameters such as pH, total solids, specific gravity, total sugar, alcohol content, absence of methanol and total phenolic content of different brands of Kumarysava samples were determined. Total sugar content and alcohol content of each brand of Kumarysava sample was found to be different. The maximum sugar content (34.65%) was found in Brand-4 and minimum (20.37%) was found in Brand-3. Similarly maximum alcohol content (8.85%) was found in Brand-2 and minimum (1.73%) was found in Brand-1 whereas in Brand-3 the alcohol content is also on the lower side (3.70%) suggesting the improper fermentation. The results are incorporated in Table-4.

Table-4: Physico-chemical properties of *Kumaryasava*

Parameter	Specification as per API	Observed value of different brands			
		Brand 1	Brand 2	Brand 3	Brand 4
pH	3.40-4.20	4.21±0.08	4.05±0.07	4.04±0.07	4.15±0.05
Total solids (%w/v)	Not Less Than 13	28.07±0.22	18.02±0.14	17.17±0.13	29.01±0.27
Specific gravity at 25°C (g/cc)	1.01-1.10	1.12±0.02	1.06±0.04	1.07±0.05	1.11±0.03
Reducing sugar (% w/v)	Not Less Than 7.5	24.76±0.24	21.07±0.28	20.37±0.36	34.65±0.41
Non-reducing sugar (% w/v)	Not More than 0.30	0.35±0.03	0.25±0.02	0.20±0.02	0.32±0.04
Alcohol content (%v/v)	5-10	1.73±0.12	8.85±0.28	3.70±0.20	7.62±0.22
Methanol	Should be absent	Absent	Absent	Absent	Absent
Total Phenolic content as Gallic acid (% w/v)	-	0.46±0.04	0.41±0.03	0.50±0.04	0.22±0.02

Microbiological Evaluations:

The total microbial plate count, yeast and mould counts of all marketed brands of *Drakshasava* 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.

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Table-5: Microbiological evaluation of different marketed brands of *Drakshasava*

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	65	<10	20	55
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
Staphyllococcus aureus	Should be absent	Absent	Absent	Absent	Absent

The total microbial plate count, yeast and mould counts of all marketed brands of *Kumaryasava* 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-6: Microbiological evaluation of different marketed brands of *Kumaryasava*

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	170	35	85	50
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
Staphyllococcus aureus	Should be absent	Absent	Absent	Absent	Absent

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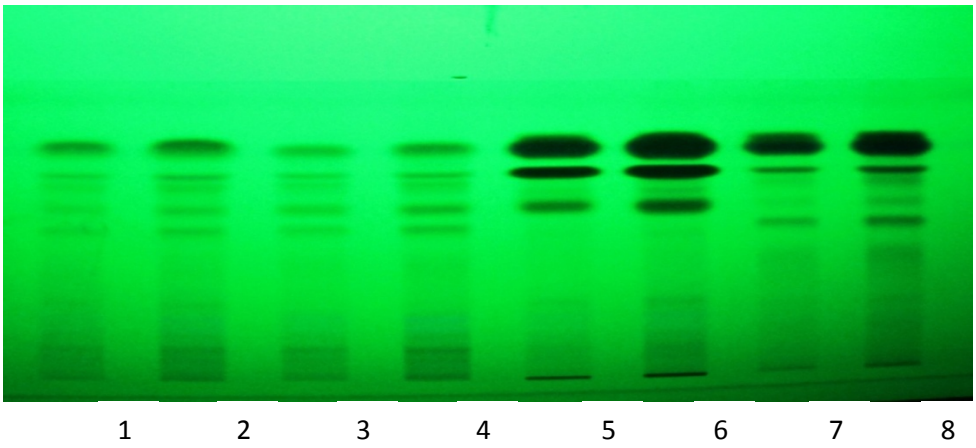
Development of TLC Fingerprinting Profile:

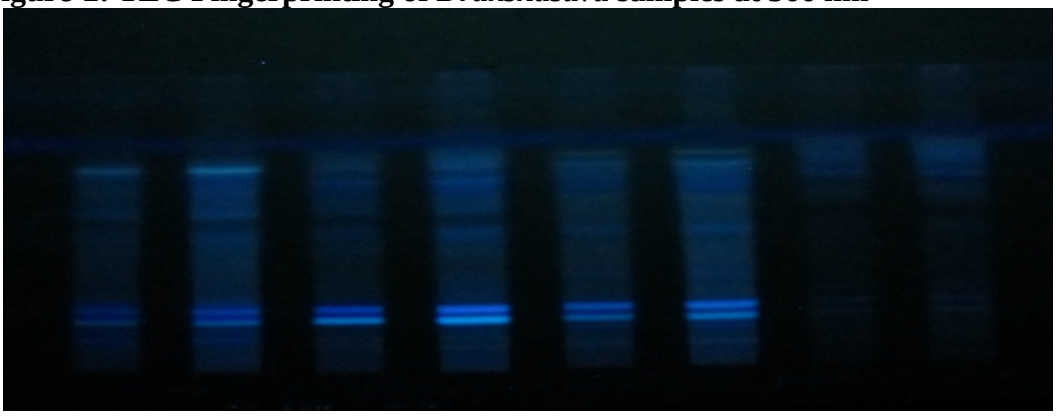
Fingerprinting is now globally accepted as a quality evaluation model of herbal medicine. So, a simple and unique TLC fingerprinting profile has been developed for all marketed brands of *Drakshasava* and *Kumaryasava* samples which could be an effective tool for the evaluation of quality as well as batch to batch consistency. *Drakshasava* samples received from Brand 1 to Brand 4 (Track No 1-8) showed similar spots (R_f 0.07, 0.22, 0.39, 0.48, 0.55, 0.63, 0.67, 0.77) with similar density at 254 nm. The results are incorporated in Figure-1.

Figure-1: TLC Fingerprinting of *Drakshasava* samples at 254 nm



Drakshasava samples received from Brand 1 to Brand 3 (Track No 1-6) showed similar spots (R_f 0.14, 0.18, 0.42, 0.53, 0.60, 0.66) with similar density at 366 nm. However, sample received from Brand 4 showed much lighter spots (Track No 7-8) at same concentration at 366 nm suggesting lesser amounts of active ingredients in the sample in comparison to other marketed samples. The results are incorporated in Figure-2.

Figure-2: TLC Fingerprinting of *Drakshasava* samples at 366 nm



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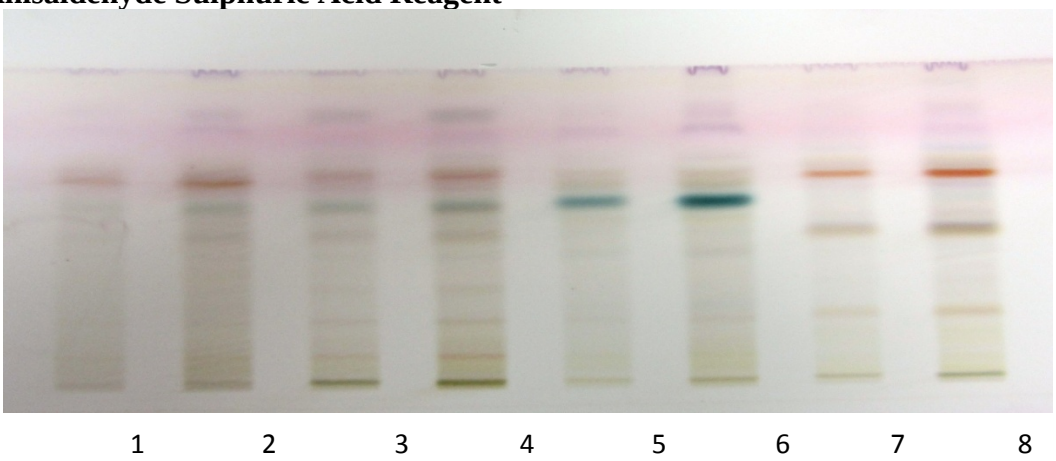
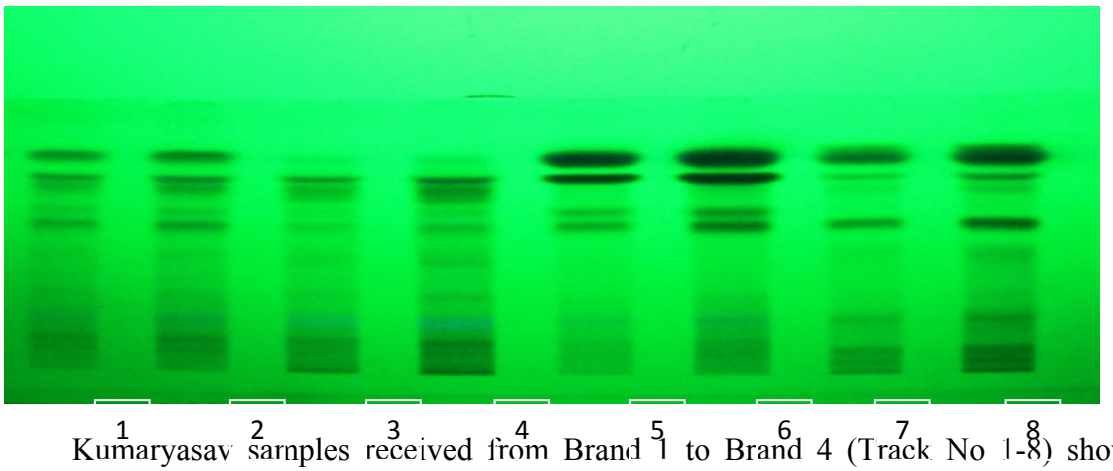
Drakshasava samples received from Brand 1 to Brand 3 (Track No 1-6) showed similar spots (R_f 0.07, 0.22, 0.39, 0.48, 0.55, 0.63, 0.67, 0.77) with similar density after derivatisation with Anisaldehyde Sulphuric Acid Reagent. However, sample received from Brand 4 showed much lighter spots (Track No 7-8) at same concentration after derivatisation with Anisaldehyde Sulphuric Acid Reagent suggesting lesser amounts of active ingredients in the sample in comparison to other marketed samples. The results are incorporated in Figure-3.

Figure-3: TLC Fingerprinting of *Drakshasava* samples after derivatisation with Anisaldehyde Sulphuric Acid Reagent



Kumaryasav 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 254nm. The results are incorporated in Figure-4.

Figure-4: TLC Fingerprinting of *Kumaryasava* samples at 254 nm



Kumaryasav 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 results are incorporated in Figure-5.

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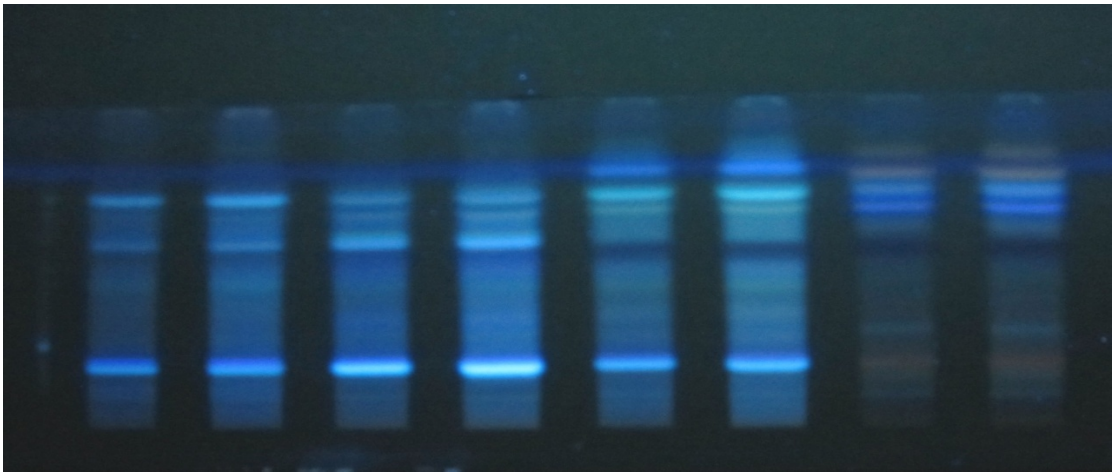
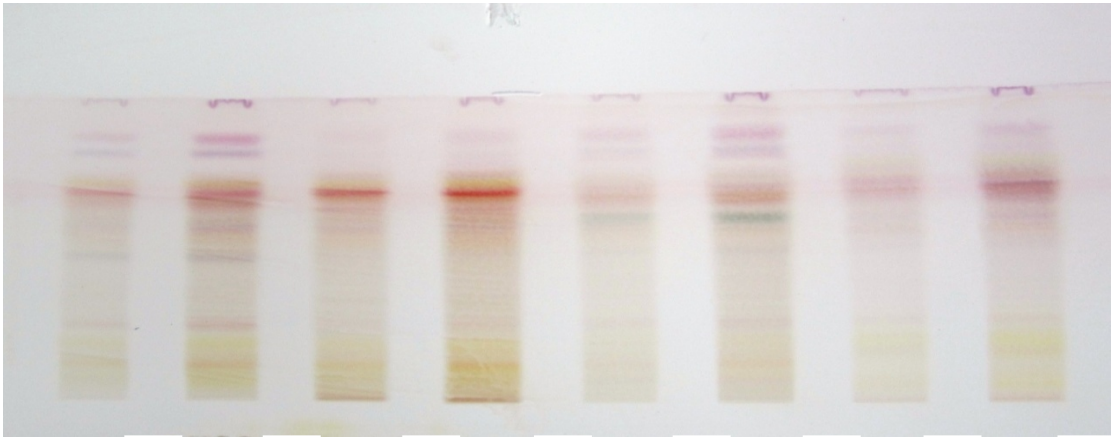
Figure-5: TLC Fingerprinting of *Kumaryasava* samples at 366 nm



Kumaryasav samples received from Brand 1 to Brand 4 (Track No 1-8) showed similar spots (R_f 0.09, 0.16, 0.26, 0.42, 0.53, 0.58, 0.65, 0.70, 0.72 with similar density after derivatisation with Anisaldehyde Sulphuric Acid Reagent. The results are incorporated in Figure-6.

Figure-6: TLC Fingerprinting of *Kumaryasava* samples after derivatisation with Anisaldehyde Sulphuric Acid Reagent



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

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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 certified [2, 5].

In the present investigation, we have evaluated four different brands of *Drakshasava* and *Kumaryasava* 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 *Drakshasava* and *Kumaryasava* 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 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.

The present investigation found lack of consistency between different brands of *Drakshasava* and *Kumaryasava* 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.

REFERENCES:

1. The Ayurvedic Pharmacopoeia of India First Edition, volume-II, Part-II, Controller of Publications Civil Lines, Delhi, 2008.
2. Kalaiselvan V, Shah AK, Patel FB, Shah CN, Kalaivani M. Quality Assessment of Different Marketed Brands of Dashmoolaristam, an Ayurvedic Formulation. International Journal of Ayurveda Research 2010; 1: 10-13.
3. WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues; 2007.
4. ISO 14502-1: 2005. Determination of Substances Characteristic of Green and Black Tea. Part 1: Content of Total Polyphenols in Tea. Colorimetric method using Folin-Ciocalteu reagent.
5. Sharma A, Shankar C, Tyagi LK, Singh M, Rao CV. Herbal Medicine for Market Potential in India: An Overview. Academic Journal of Plant sciences 2008; 1: 26-36.