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COMPARATIVE STUDY OF *ALOE VERA* LEAF & MARKETED *ALOE VERA* JUICE BY HPTLC

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ABSTRACT

Aloe vera gel and *Aloe vera* juice is out there in the market nowadays and is preferred by numerous customers. This study is oriented towards standardization of Barbaloin present in *Aloe. vera* gel. And then taking *Aloe vera* gel as reference standard for standardising *Aloe vera* juice. Barbaloin, was separated by chromatographic method using gradient elution technique and was determined by using HPTLC- photo densitometric scanner. The proposed method is easy, sensitive, cost effective and may be used for the regular assay of Barbaloin in phytomedicines containing *Aloe. vera* gel and qualitative assay of *Aloe vera* juice. Other qualitative tests just like the presence of heavy metals & the Microbiological assay of the *A. vera* gel juice was also performed.

KEYWORDS: *Aloe vera*, Barbaloin, Silica Gel, Gradient, Photo densitometry, Phyto medicines, Heavy Metal, Microbiological Assay.

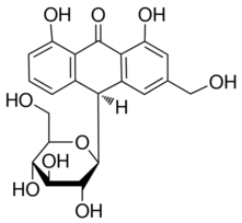
1. INTRODUCTION

Aloe vera has been traditionally used to treat skin injuries (burns, cuts, insect bites, and eczemas) and digestive problems because its anti-inflammatory, antimicrobial, and wound healing properties. The most investigated active compounds are aloe-emodin, aloin, aloesin, emodin, and acemannan.[1]

Aloe vera (*Aloe barbadensis* Miller, family Xanthorrhoeaceae) is a perennial green herb with bright yellow tubular flowers that is extensively distributed in hot and dry areas of North

Africa, the Middle East of Asia, the Southern Mediterranean, and the Canary Islands. *Aloe vera* derives from "Allaeh" (Arabic word that means "shining bitter substances") and "Vera" (Latin word that means "true"). The colorless mucilaginous gel from *Aloe vera* leaves has been extensively used with pharmacological and cosmetic applications. Traditionally, this medicinal plant has been employed to treat skin problems (burns, wounds, and anti-inflammatory processes). Moreover, *Aloe vera* has shown other therapeutic properties including anticancer, antioxidant, antidiabetic, and antihyperlipidemic. *Aloe vera* contains more than 75 different compounds, including vitamins (vitamin A, C, E, and B12), enzymes (i.e., amylase, catalase, and peroxidase), minerals (i.e., zinc, copper, selenium, and calcium), sugars (monosaccharides such as mannose-6-phosphate and polysaccharides such as glucomannans), anthraquinones (aloin and emodin), fatty acids (i.e., lupeol and campesterol), hormones (auxins and gibberellins), and others (i.e., salicylic acid, lignin, and saponins).[2,3,4]

2. Chemical Structure



Molecular Formula $C_{21}H_{22}O_9$

Molecular Weight 418.4

IUPAC Name

(10*S*)-1,8-di hydroxy-3-(hydroxyl methyl)-10- [(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-tri hydroxy-6 (hydroxyl methyl) oxan-2-yl] - 10*H*-anthracen-9-one

3. MATERIALS & METHODS

3.1. Samples under Test

Sl. No.	Sample Type	Sample Name
1	Raw Material	*Aloe vera* leaf gel
2	Product (Sample)	*Aloe vera* Juice

3.2. Determination Of Heavy Metal

3.2.1. Determination of Lead (Pb) (Graphite Oven Method)[5]

3.2.1.1. Determination conditions: Reference condition: dry temperature: 100-1200, maintain 20 seconds; ash temperature: 400-750, maintain 20-25 seconds; measurement wavelength: 283.3 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

3.2.1.2. Preparation of lead standard stock solution: Measure accurately a quantity of lead single-element standard solution to prepare standard stock solution with 2 per cent nitric acid solution, which containing 1 μg per ml, stored at 0-50.

3.2.1.3. Preparation of calibration curve: Measure accurately a quantity of lead standard stock solutions respectively, diluted with 2 per cent nitric acid solution to the concentration of 0, 5, 20, 40, 60, 80 ng per ml, respectively. Measure respectively accurately 1 ml the above solution, add respectively 1 ml of 1 per cent ammonium dihydrogen phosphate and 0.2 per cent *magnesium nitrate* mix well, pipette accurately 20 μl to inject into the atomic generator of graphite oven and determine their absorbance, then draw the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

3.2.1.4. Preparation of test solution- Method

Weigh accurately 0.5 g of the coarse powder of the substance being examined, transfer into a casparian flask, add 5-10 ml of the mixture of *nitric acid* and *perchloric acid* (4 : 1), add a small hopper on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-black, add again a quantity of the above mixture, continuously heat till the solution becomes clean and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colourless and transparent or a little yellow, cool, transfer it into a 50 ml volumetric flask, wash the container with 2 per cent *nitric acid solution* add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

3.2.1.5. Determination: Measure accurately 1 ml of the test solution and its corresponding reagent blank solution respectively, add 1 ml of solution containing 1per cent *ammonium dihydrogen phosphate* and 0.2 per cent *magnesium nitrate*, shake well, pipette accurately 10-20 μl to determine their absorbance according to the above method of "Preparation of calibration curve". Calculate the content of lead (Pd) in the test solution from the calibration curve.

3.2.2. Determination of Cadmium (Cd) (Graphite Oven Method)[6]

3.2.2.1. Determination conditions: Reference condition: dry temperature: 100-1200, maintain 20 seconds; ash temperature: 300-5000 , maintain 20-25 seconds; atomic temperature: 1500-19000, maintain 4-5 seconds; measurement wavelength: 228.8 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

3.2.2.2. Preparation of Cd standard stock solution: Measure accurately a quantity of Cd single-element standard solution to prepare standard stock solution Cd with 2 per cent nitric acid, which containing 0.4 µg per ml Cd, stored at 0-50.

3.2.2.3. Preparation of calibration curve: Measure accurately a quantity of cadmium standard stock solutions, diluted to the concentration of 1.6, 3.2, 4.8, 6.4 and 8.0 ng per ml with 2 per cent nitric acid, respectively. Pipette accuratel 10 µl the above solutions respectively, inject them into the graphite oven, determine their absorbance, and then draw the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

3.2.2.4. Preparation of test solution- Method

Weigh accurately 0.5 g of the coarse powder of the substance being examined, transfer into a casparian flask, add 5-10 ml of the mixture of *nitric acid* and *perchloric acid* (4 : 1), add a small hopper on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-black, add again a quantity of the above mixture, continuously heat till the solution becomes clean and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colourless and transparent or a little yellow, cool, transfer it into a 50 ml volumetric flask, wash the container with 2 per cent *nitric acid solution* add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

3.2.2.5. Determination*:* Pipette accurately 10-20 µl of the test solution and its corresponding reagent blank solution respectively, determine their absorbance according to the above method of "Preparation of calibration curve. If interference occurs, weigh accurately respectively 1 ml of the standard solution, blank solution and test solution,add 1 ml of a solution containing 1per cent *ammonium dihydrogen phosphate* and 0.2 per cent *magnesium nitrate*, shake well, determine their absorbance according to the method above, calculate the content of Cd in the test solution from the calibration curve.

3.3. Total Aerobic Microbial Count

3.3.1. Pre-treatment of Sample[7]

Dissolve 10 g of the sample in buffered sodium chloride-peptone solution *p*H 7.0 and adjust the volume to 100 ml with the same medium. If necessary, adjust the *p*H to about 7.

3.3.2. Plate count for bacteria: Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pre-treated preparation and about 15 ml of liquefied *casein soyabean*

digest agar at not more than 450. Alternatively, spread the pre-treated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pre-treated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 300 to 350 for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.[8]

3.3.3. Plate count for fungi: Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pre-treated preparation and about 15 ml of liquefied *Sabouraud dextrose agar with Antibiotics* at not more than 450. Alternatively, spread the pre-treated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pre-treated preparation as described above so that a colony count of not more than 100 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 200 to 250 for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 100 colonies per plate as the maximum consistent with good evaluation.[9]

3.4. Standardisation By HPTLC

Plate Details
Plate Size X×Y- 5.0 × 10.0 cm Material- HPTLC Plate Silica Gel 60 F 254. Manufacturer- E. Merck KGaA
Instrument Details
Software- WINCATS Planer Chromatography Manager Sample Application-CAMAG Linomat 5 Instrument- CAMAG Linomat 5 “Linomat 5_160424” S/N- 160424 (1.00.12)
Linamoat 5 Application Parameter
Spray Gas- Inert Gas Sample Solvent Type- Water Dosage Speed- 50 nl/s Pre-dosage Vol.- 0.2 µl
Sequence
Syringe Size- 100 µl No. of Tracks- 2 Application Position Y- 10.0 mm Band Length- 8 0 mm
Development- Glass Tank

Chamber Type- Twin Through Chamber 20*10 cm Mobile Phase- Ethyl Alcohol : Methanol : Water- 10:2:1 Solvent Front Position- 88.0 mm Volume- 10.0 ml Drying Device- Hair Dryer Temperature- 60°C Time Notes- 5 min. Detection- CAMAG TLC Scanner 3 No. of Tracks- 2 Position of First Track X- 18.0 mm Distance between Tracks- 14.0 mm Scan Start Position Y- 5.0 mm Scan end Position Y- 95.0 mm Slit Dimensions- 5.00* 0.45 mm, Micro Optimize Optical System- Light Scanning Speed- 20 mm/s Data Resolution- 100 µm/step
Measurement Table
Wavelength- 340 Lamp- D2 Measurement Type- Remission Measurement Mode- Absorption Optical Filter- 2nd Order Detector Mode- Automatic PM High Voltage- 329 V
Executed By
RKM Quality Testing Laboratory, Ramkrishna Mission Ashram, Narendrapur, Kolkata-700103.

Sl. No.	Appl. Position	Appl. Vol.	Vial #	Sample ID	Active
1	18.0 mm	10.0 µl	1	Aloe Ethanolic Ext.	Yes
2	32.0 mm	10.0 µl	2	Aloe Juice	Yes

3.5. Sample Preparation

3.5.1. Aloe Ethanolic Extract- *Aloe vera* gel was extracted with ethanol in a volumetric flask. Sonicate for 15 mins.

3.5.2. *Aloe vera* Juice was directly applied to the applicator.

4. RESULTS & DISCUSSION

Table-1: Test Report for Heavy Metals.

Heavy Metal Analysed	Heavy Metal Content in *Aloe vera* Juice (ppm)	Recommended Limit (ppm)
Lead	0.19	10.0
Cadmium	0.02	0.3

Table-2: Test Report for Microbial Contaminants.

Microbial Contaminants Tested	*Aloe vera* Juice	Recommended Limit
Total Viable Aerobic Count	Below Detection Limit	1×10^5 CFU/gm
Total Fungal Count	Below Detection Limit	1×10^3 CFU/gm

4.1. Test Report for HPTLC Analysis

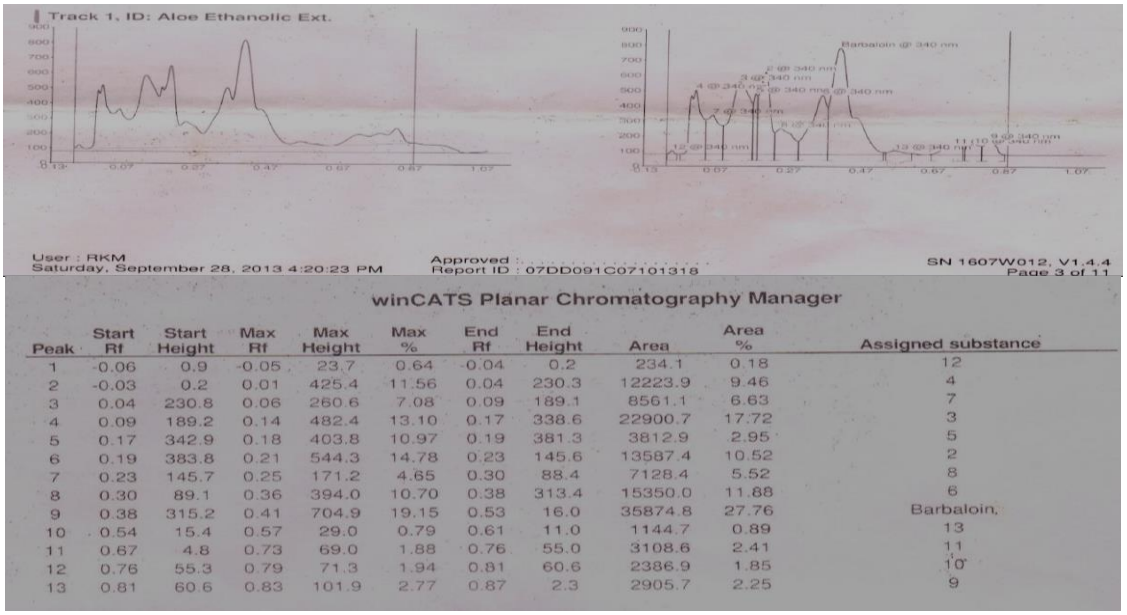
Ethanolic extract of *Aloe vera* leaf gel & *Aloe vera* juice was subjected to HPTLC analysis for the qualitative determination of Barbaloin to determine whether it is present in both the sample by comparison of their Rf values respectedly.

The results shows the Rf value of Barbaloin (0.41) & also more Rf values (Approximately 12) is obtained. All the Rf values are shown in Fig .-3. in this study emphasis is given only on the Bio active molecule present in *Aloe vera* 'Barbaloin'.

The chromatograms generated for 2 samples, viz. Aloe Ethanolic Ext. & Aloe Juice are given below in Fig -1 & Fig -2 respectively.

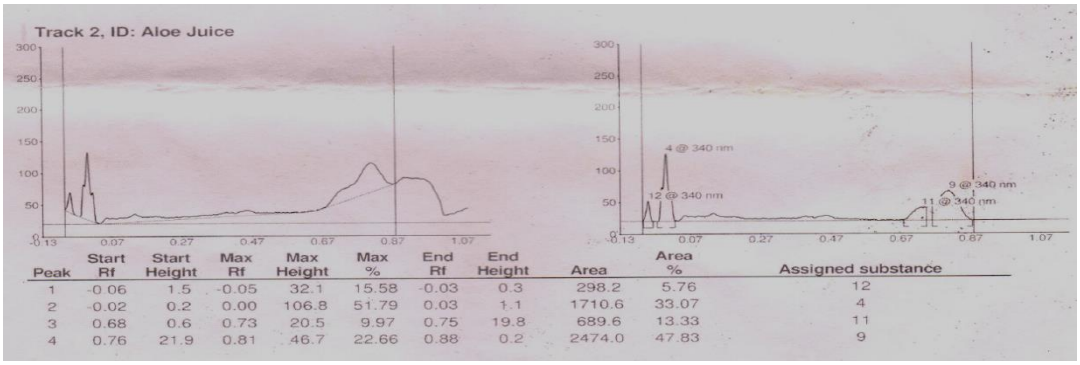
The Chromatogram of Barbaloin on both the tracks are shown in Fig .4.

The other Rf values in both the samples viz. Aloe Ethanoic ext. & *Aloe vera* juice are 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 named accordingly, & the chromatograms obtained from both the tracks are given below in Fig -5, Fig -6, Fig -7, Fig -8, Fig -9, Fig -10, Fig -11, Fig -12, Fig -13, Fig -14, Fig -15, Fig -16 respectively with their Rf values herewith.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.06	0.9	-0.05	23.7	0.64	-0.04	0.2	234.1	0.18	12
2	-0.03	0.2	0.01	425.4	11.56	0.04	230.3	12223.9	9.46	4
3	0.04	230.8	0.06	260.6	7.08	0.09	189.1	8561.1	6.63	7
4	0.09	189.2	0.14	482.4	13.10	0.17	338.6	22900.7	17.72	3
5	0.17	342.9	0.18	403.8	10.97	0.19	381.3	3812.9	2.95	5
6	0.19	383.8	0.21	544.3	14.78	0.23	145.6	13587.4	10.52	2
7	0.23	145.7	0.25	171.2	4.65	0.30	88.4	7128.4	5.52	8
8	0.30	89.1	0.36	394.0	10.70	0.38	313.4	15350.0	11.88	6
9	0.38	315.2	0.41	704.9	19.15	0.53	16.0	35874.8	27.76	Barbaloin.
10	0.54	15.4	0.57	29.0	0.79	0.61	11.0	1144.7	0.89	13
11	0.67	4.8	0.73	69.0	1.88	0.76	55.0	3108.6	2.41	11
12	0.76	55.3	0.79	71.3	1.94	0.81	60.6	2386.9	1.85	10
13	0.81	60.6	0.83	101.9	2.77	0.87	2.3	2905.7	2.25	9

Fig.-1:



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.06	1.5	-0.05	32.1	15.58	-0.03	0.3	298.2	5.76	12
2	-0.02	0.2	0.00	106.8	51.79	0.03	1.1	1710.6	33.07	4
3	0.68	0.6	0.73	20.5	9.97	0.75	19.8	689.6	13.33	11
4	0.76	21.9	0.81	46.7	22.66	0.88	0.2	2474.0	47.83	9

Fig.-2

Substance name	Rf	Window size
Barbaloin	0.41	0.500
2	0.21	0.500
3	0.14	0.500
4	0.01	1.100
5	0.18	0.500
6	0.36	0.500
7	0.06	0.500
8	0.25	0.500
9	0.82	1.900
10	0.79	0.500
11	0.73	0.500
12	-0.05	0.500
13	0.57	0.500

Fig .-3

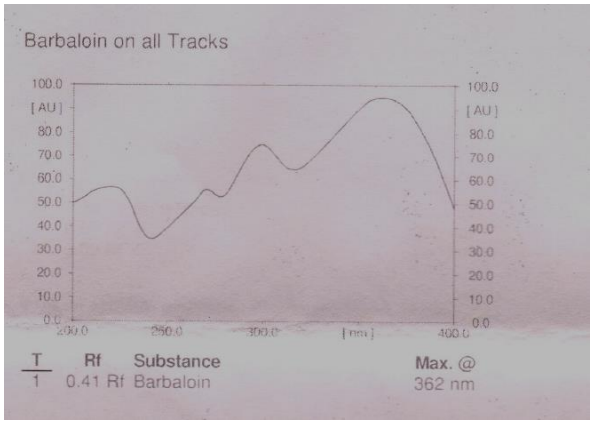


Fig.- 4

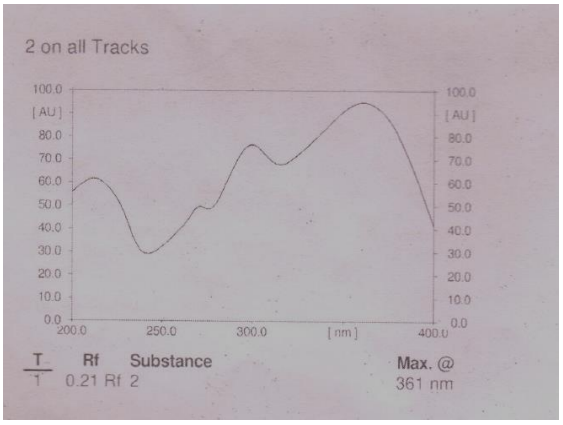


Fig.- 5

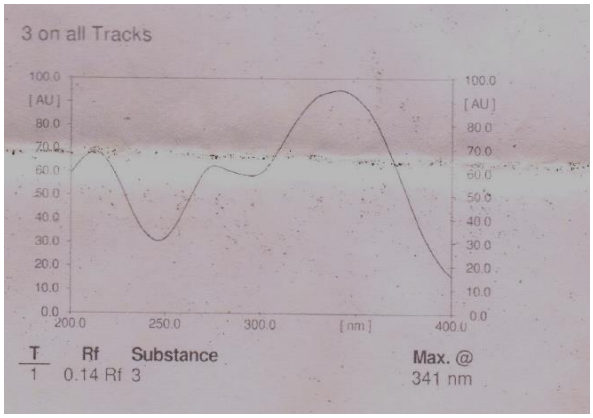


Fig.- 6

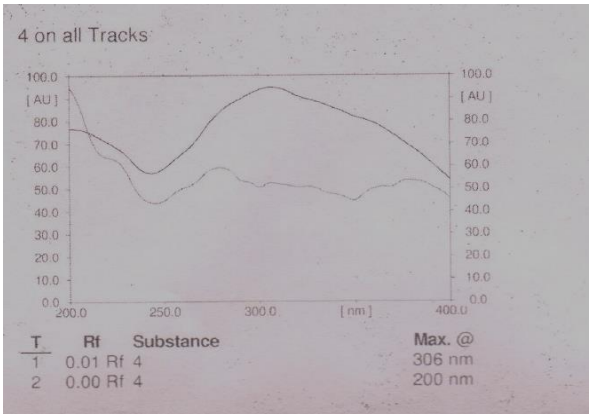
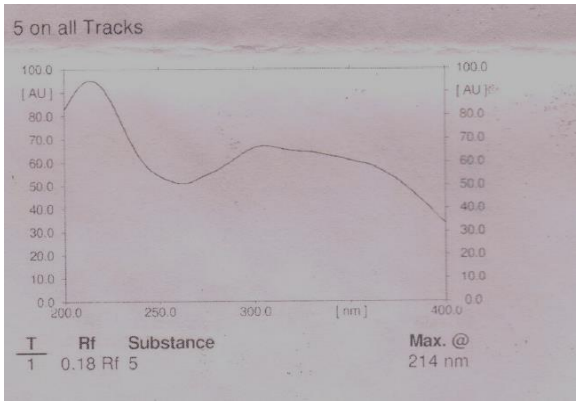


Fig.- 7



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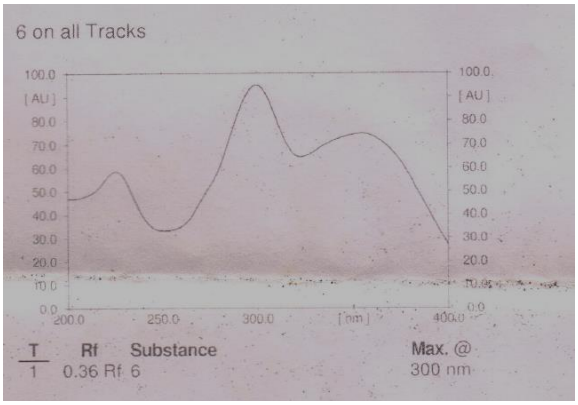

Fig.- 9

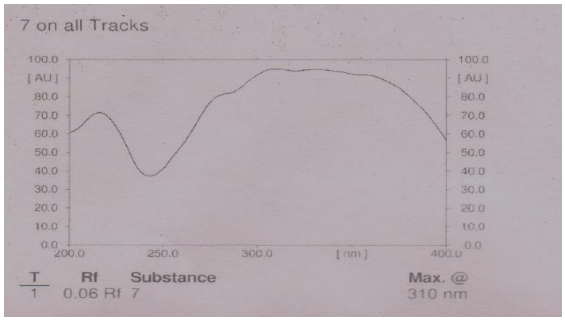

Fig.- 10

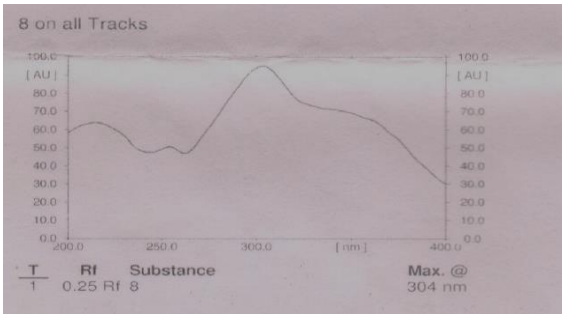

Fig.- 11

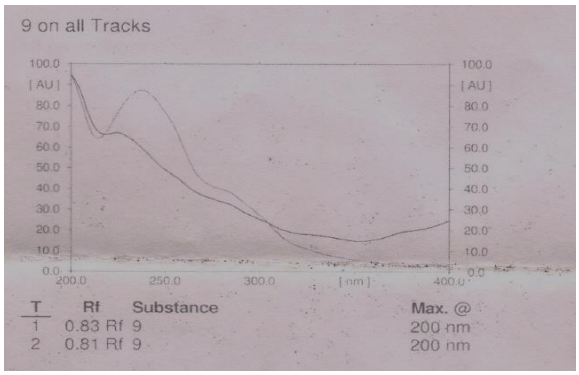

Fig.- 12

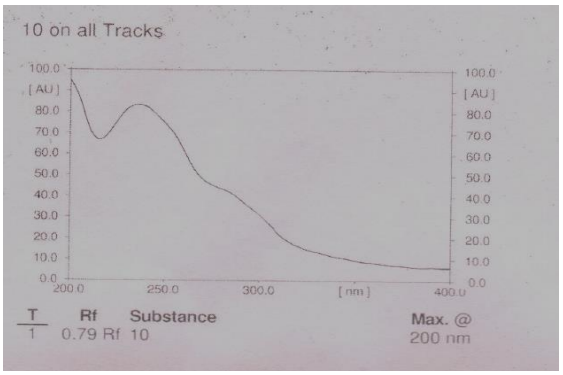

Fig.- 13

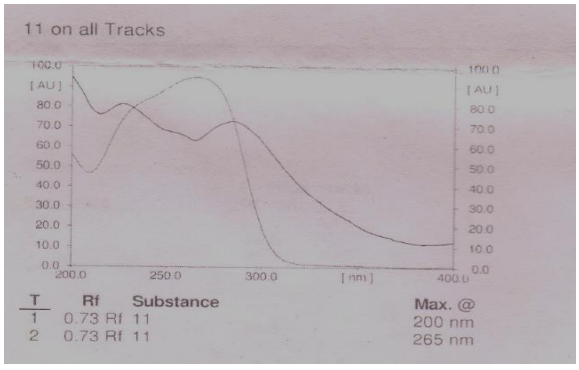

Fig.- 14

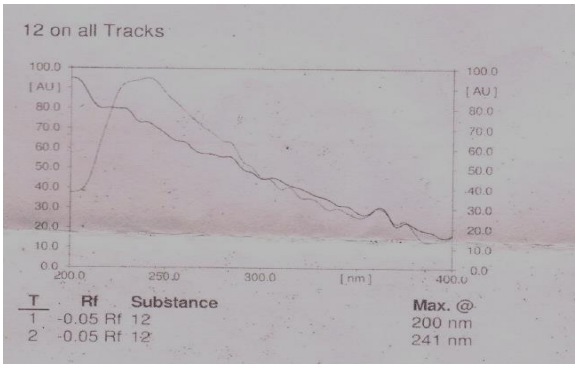

Fig.- 15

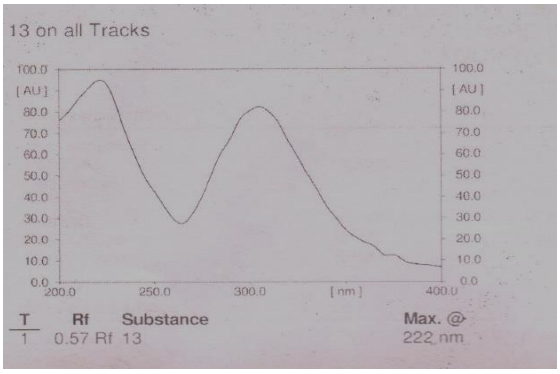

Fig.- 16

DISCUSSION

In order to identify the reliable quality control marker of *Aloe vera*, some extensive studies has been done regarding barbaloin. These studies revealed that barbaloin is present in considerable quantities in both *Aloe vera* leaf gel and *Aloe vera* juice. Hence HPTLC based qualitative studies have been done on *Aloe vera* gel as standard and its barbaloin as biomarker. The *Aloe vera* gel is then used as a standard for determination of quality of commercially available *Aloe vera* juice. Also emphasis has been given on the heavy metal content and microbial limit of commercially available *Aloe vera* juice. The quantity of lead and cadmium content was found was to be within permitted limit (table-1), also the microbial limit of viable aerobic bacteria and fungi was found to be satisfactory (table-2). The proposed method is simple and sensitive and can be used for the routine assay of Barbaloin in phytomedicines containing *Aloe vera* gel. The details are quite evident. This can be a good basis of reviewing the commercially marketed *Aloe vera* juices, so the consumer can be benefited. Also it provides a comparative low cost method. The present HPTLC method is rapid, simple and accurate for qualitative monitoring of barbaloin in *Aloe vera* plant and its commercial product.

CONCLUSION

The potent cathartic compound of *Aloe vera* viz. Barbaloin was isolated and characterized by chromatographic and spectroscopic methods. In order to identify the reliable marker for quality control, they have been subjected to detailed HPTLC studies. The HPTLC quantification studies reveal that barbaloin is present in both *Aloe vera* leaf gel and *Aloe vera* juice. Hence it can be taken as a reliable quality control marker of *Aloe vera* and its commercial formulations. The present HPTLC method is rapid, simple and accurate for quantitative monitoring of *Aloe vera* plant and its commercial product with respect to

barbaloin. However further studies are required to confirm this findings and develop more suitable methods accordingly.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors have no Conflict of Interest.

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