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Biological and physico-chemical study of berg-e-kasondi

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Abstract
The impending world is reliant on traditional medicine, and its recognition escalating at present as essential. In regulating the therapeutic efficacy of herbal drugs, standardization and quality control are the key factors. Organoleptic characters are not much consistent in establishing the standards of herbal drugs, for which the instrumental analysis of the drugs provide more accurate picture regarding the qualitative and quantitative aspects of bioactive molecules, which are held liable for therapeutic action and is widely accepted in quality assessment of herbal drugs. The present study was taken to scientifically evaluate the sample by various parameters such as macroscopy, microscopy, powder study and physico-chemical analysis such as loss on drying, extractive values i.e., water-soluble matter, ethanol soluble matter, Hexane soluble matter, percentage of ash values i.e., Total ash and acid insoluble ash, pH of 1% & 10% aqueous solution and HPTLC Fingerprinting analysis. This will be helpful for botanical identification and standardization of Kasondi leaves in crude form.

Keywords: Morphological, microscopical, physicochemical, standardization

Introduction
The drug Kasondi consists of dried leaves of *Cassia occidentalis* L. of family - Fabaceae. Plant Kasondi is a much branched, smooth, half woody herb or shrub about 0.8 to 1.8 m tall. Plant is distributed throughout India and the tropics generally. Its other names are Kalkasunda in Bengali, Kanuvai in Guajarati, Nattandagarai, Peyavirai, Ponnavarai in Tamil, Natrum Takara, Ponnaveeram in Malayalam, Kasinda in Telugu [4, 7].
The leaves of this plant are tasty; aphrodisiac, alexiteric; cure cough, asthma, stomachic cure, good for sore throat and biliousness. In the West Indies, the root is considered diuretic and the leaves taken internally and applied externally are given in case of itch and other cutaneous diseases. In Guinea every part of the plant is considered tonic and febrifuge. The fresh leaves are ground and applied to wounds and swellings. The leaves are boiled and the decoction drunk by children to cure worms. The whole plant is purgative, tonic and febrifuge. The seeds and leaves are used externally in cutaneous disease [1, 6, 7].
The present study was taken up to scientifically evaluate by the various physico-chemical parameters such as loss on drying, extractive values i.e., water-soluble matter, ethanol soluble matter, Hexane soluble matter, percentage of ash values i.e., Total ash and acid insoluble ash, pH of 1% & 10% aqueous solution and HPTLC fingerprinting analysis to identify various chemical components present in plant material. Similar study has been published for other Unani drugs [8, 11].

Material and Methods
I. Collection and Authentication of plant Material
The leaves of *Cassia occidentalis* were collected from Herbal Garden PCIM&H Campus. The plant material was identified and authenticated by the botanist of DSRI, Ghaziabad.

II. (a) Pharmacogenetic Studies
Pharmocognostical studies were carried out as per the Ayurvedic Pharmacopoeia and Unani Pharmacopeia of India [2, 3].

II. (b) Powder Microscopy
10-15 grams of compound drug was taken and stirred carefully with hot water in a beaker; the residue was discarded and the process was repeated; then small amount of sediment was taken

in a slide and mounted with glycerine; then a small amount of residue was taken and treated separately with chloral hydrate and washed with distilled water and mounted with glycerine and different characters were observed under the microscope [13].

III. Physicochemical analysis

The physico-chemical methods *viz*., loss on drying, ash values, solubility in different solvents, pH values of 1% & 10% aqueous solution etc. are useful tools in standardization of a herbal product for maintaining the batch-to-batch consistency. The drug samples were subjected for the standardization of physicochemical parameters and analysed as per the standards method [5].

IV. Thin Layer Chromatography

a) Preparation of extracts of the sample drug

The drug sample of 2 g was extracted with 40 ml of alcohol and chloroform separately in boiling tubes by ultrasonic sonicator for 30 min at 600 C. The extract was filtered and concentrated to 5 ml and carried out the thin layer chromatography. Alcoholic and Chloroform extracts were spotted on silica gel "G" plate by semi-Automatic Applicator and developed with Toluene: Ethyl Acetate: Formic Acid (9:1:0.5) as mobile phase. Thin layer chromatography fingerprint profiling has been carried out in triplicate.

b) Development and determination of the solvent system

The sample extracts are spotted as 10 mm band on Pre-coated Aluminium Sheets of Silica Gel 60 F254 (Merck). After trying with various solvent system with variable volume ratios, the suitable solvent system as Toluene: Ethyl Acetate: Formic Acid (9:1:0.5) was selected in its proportional ratio and developed in the Twin through TLC chamber to the maximum height of the plate so that components are separated on the polar phase of silica gel and mobile phase of solvent system.

c) Detection system

After the developing, the TLC plate was dried completely and detected under the UV visible chamber at366nm & 254nm and also by derivatization with 1% Vanillin-sulphuric acid and heated at 1050C for 5minutes and then observed in the UV chamber for detection of spots at 560nm as shown in figure 1.

d) HPTLC instrumental conditions

HPTLC was performed on 10 cm × 10 cm Pre-coated Aluminium Sheets of Silica Gel 60 F254 (Merck). Samples solution of about 10µl were applied as 10 mm width bands using Semi-Automatic TLC applicator system of the CAMAG Linomat 5. A Linear ascending development with Toluene: Ethyl Acetate: Formic Acid (9:1:0.5) as mobile phase was carried out in a twin trough glass chamber previously saturated with mobile phase vapour for 20 minutes at room temperature (25±2 °C). The development of solvent distance was 80 mm. After development plates were air- dried. TLC plate was scanned by CAMAG TLC SCANNER 4 at 366, 254 and 580 nm wavelength and operated by Vision CATS 3.1 version software. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190-600 nm. The slit dimensions were 4 mm × 6 mm [10, 12, 14]

Results

A) Macroscopic

The leaf is green on the adaxial surface and light green on the abaxial surface. It has a characteristic smell. The lamina is broadly lanceolate to ovate with an acute apex. It is 4-9 cm long and 1.5-3 cm broad with an entire margin and an obtuse base bearing a gland at the base of the leaf rachis. The venation is reticulate. The leaf is a compound pinnate with 4-5 pairs of leaves. The leaf has a glabrous surface (Fig 1).

B) Microscopic

Leaflet: T.S. of leaflet shows following structures- Both the upper and lower epidermis have thick-walled unicellular covering trichomes. Epidermal cells are polygonal in shape; in both the epidermis paracytic stomata may be seen. Prismatic crystals of calcium oxalate are present. Cluster of crystals are present in the palisade and spongy parenchyma. In the midrib region, epidermis has thick cuticle. Two to three layers of schlerenchymatous fibres are present in vascular bundle. In midrib region lower epidermis is followed by two to three layers of collenchyma. (Fig-2, 3).

C) Powder

Green, odour and taste not characteristics; Powder microscopy of the leaves revealed the presence of crystal fibre, vessels with spiral thickenings, paracytic stomata and wavy epidermal cells, unicellular trichomes. (Fig-4-7).

Macroscopic

Fig 1: Berg-e-Kasondi

Photomicrographs

Microscopic

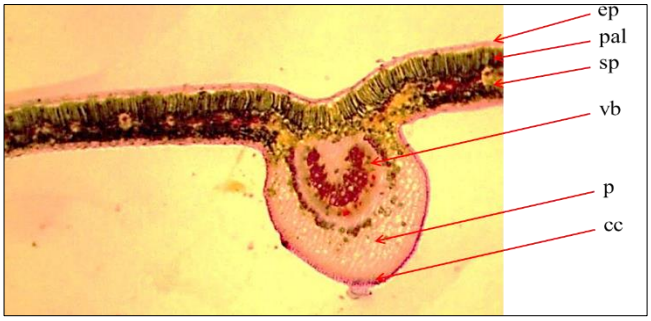


Fig 2: Leaflet T.S. 4X

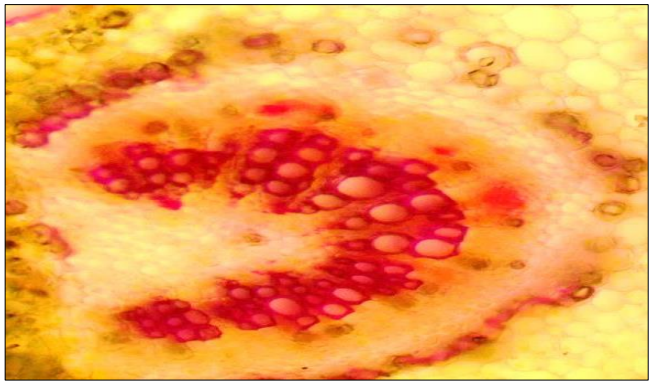


Fig 3: T.S. of midrib 20X

Abbreviations

e.p. = epidermis, pal = palisade cells, v.b. = vascular bundle, s.p. = spongy parenchyma, c.c. = collenchyma cells, p.c. = parenchyma cells

Powder study

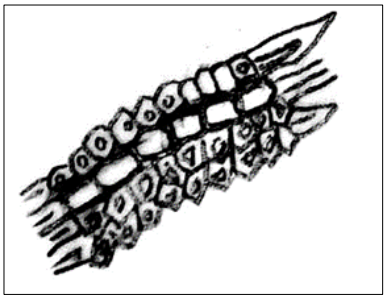


Fig 4: Crystal fibre

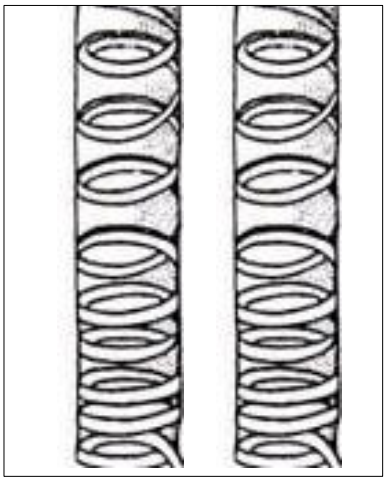


Fig 5: Spiral Vessels

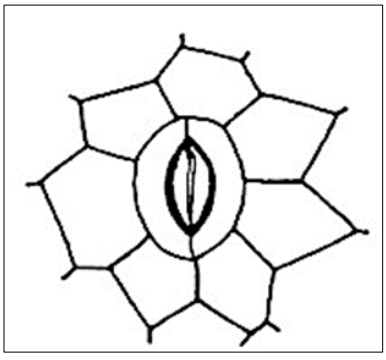


Fig 6: Epidermis with stomata.

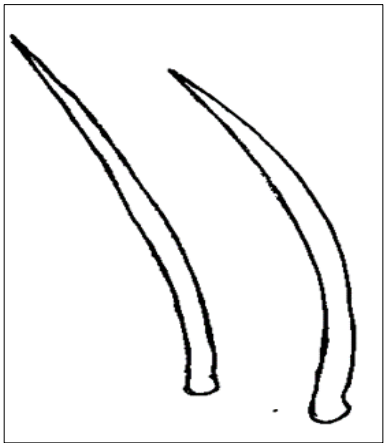


Fig 7: Unicellular trichomes

D.) Physicochemical analysis

Table 1: Physico chemical parameters of Berg-e-Kasondi

S. No.	Parameter	Values
1.	Foreign matter (%)	1.60
2.	Loss in Weight on drying at 105 °C (%)	8.92
3.	Total ash (%w/w)	9.50
4.	Acid insoluble ash (%w/w)	0.35
5.	Ethanol soluble Extractive (%)	12.20
6.	Water soluble matter (%w/w)	32.65
7.	Hexane soluble Extractive (%)	5.25
8.	pH of 1% aqueous solution	7.67
9.	pH of 10% aqueous solution	7.93

E.) HPTLC Profile

a) High Performance Thin Layer Chromatography of Chloroform extract

TLC profile under UV 254 nm showed three major peaks at Rf values 0.025, 0.301 & 0.994 and the under UV 366 nm three major peaks at 0.245, 0.322 & 0.583 Rf values and under visible region after derivatization with 1% Vanillin-Sulphuric acid showed seven spots at Rf values 0.074, 0.120, 0.193, 0.307, 0.398, 0.835 & 0.991 on TLC plate. (Fig-8-25).

TLC of Chloroform extract of Berg-e-kasondi

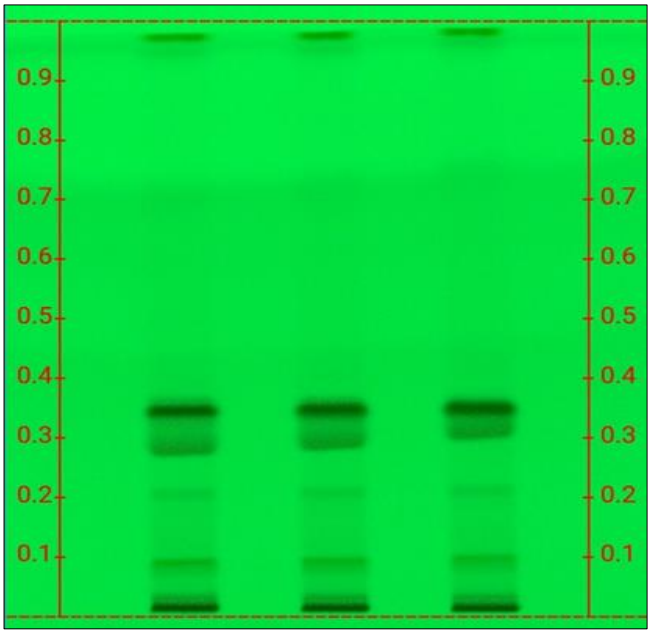


Fig 8: B-I B-II B-III At 254 nm

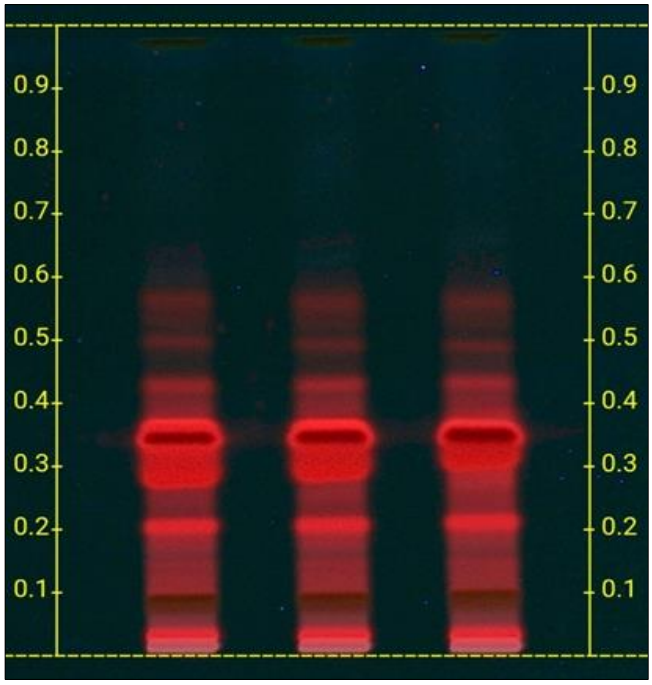


Fig 9: B-I B-II B-III At 366 nm

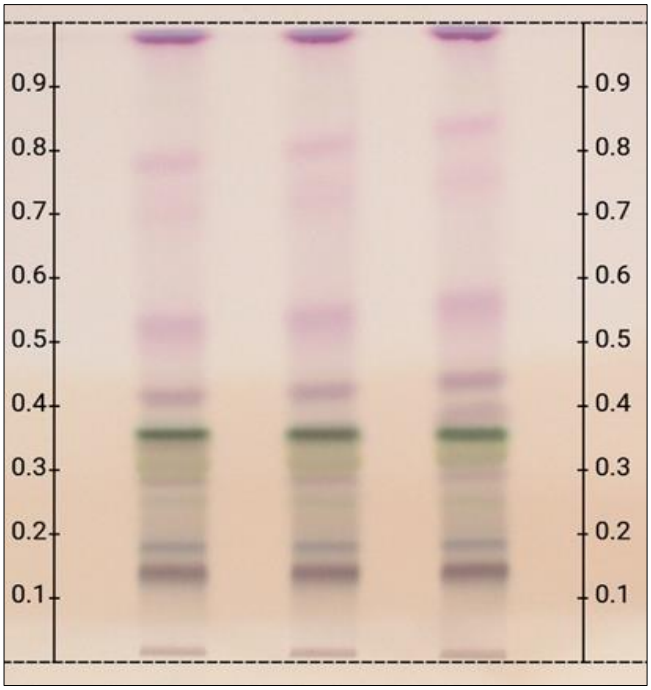


Fig 10: B-I B-II B-III At 560 nm after derivatization with VSA

4 peak(s) found

#	Max R_F	Height	Height %	Start R_F	Start height	End R_F	End height	Area	Area %	Assigned to
1	-0.046	0.0215	3.65	-0.050	0.0000	-0.035	0.0000	0.00013	0.83	-- Not assigned --
2	0.025	0.0925	15.71	0.014	0.0588	0.045	0.0033	0.00158	10.45	-- Not assigned --
3	0.301	0.2707	46.00	0.276	0.1551	0.332	0.0292	0.01001	66.11	-- Not assigned --
4	0.994	0.2038	34.64	0.980	0.0946	1.009	0.0000	0.00342	22.61	-- Not assigned --

Integration

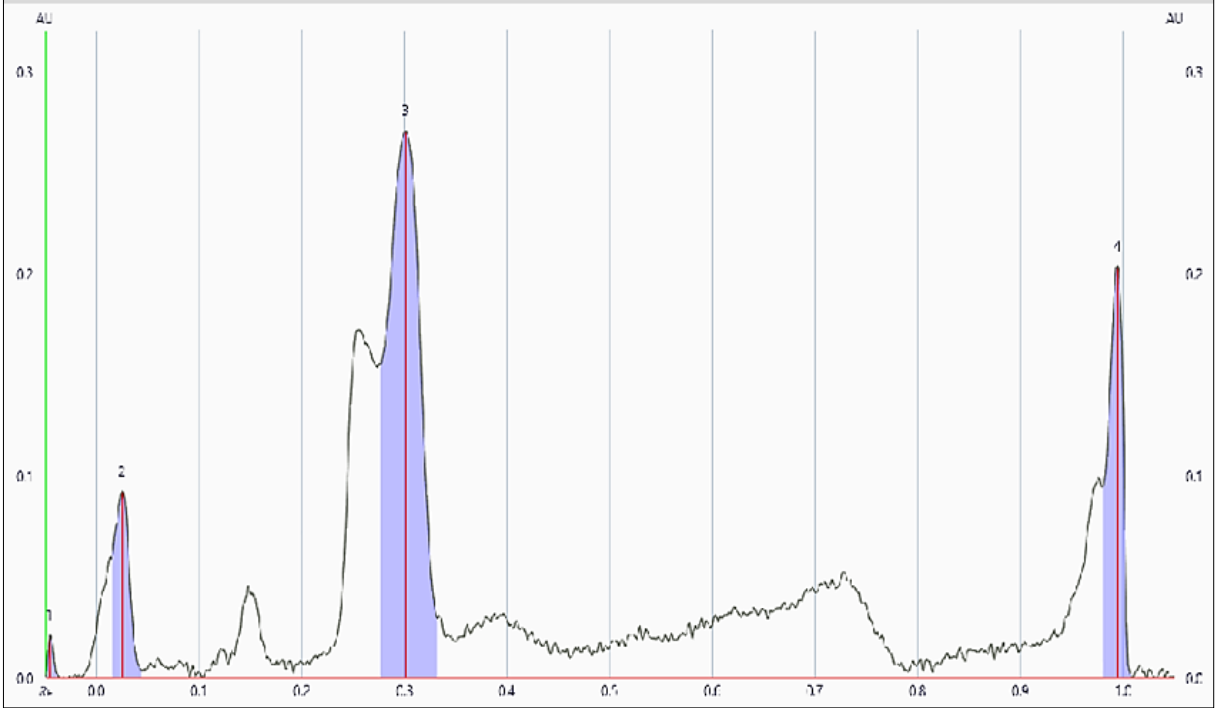


Fig 11

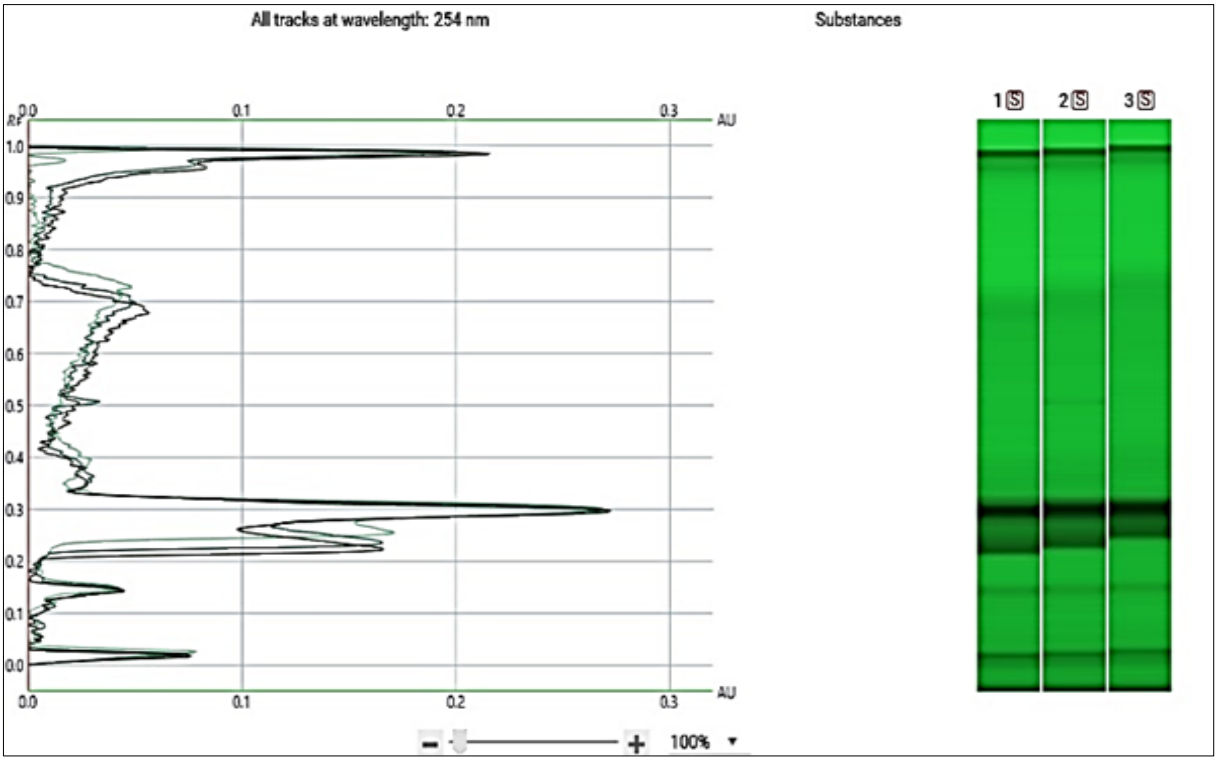
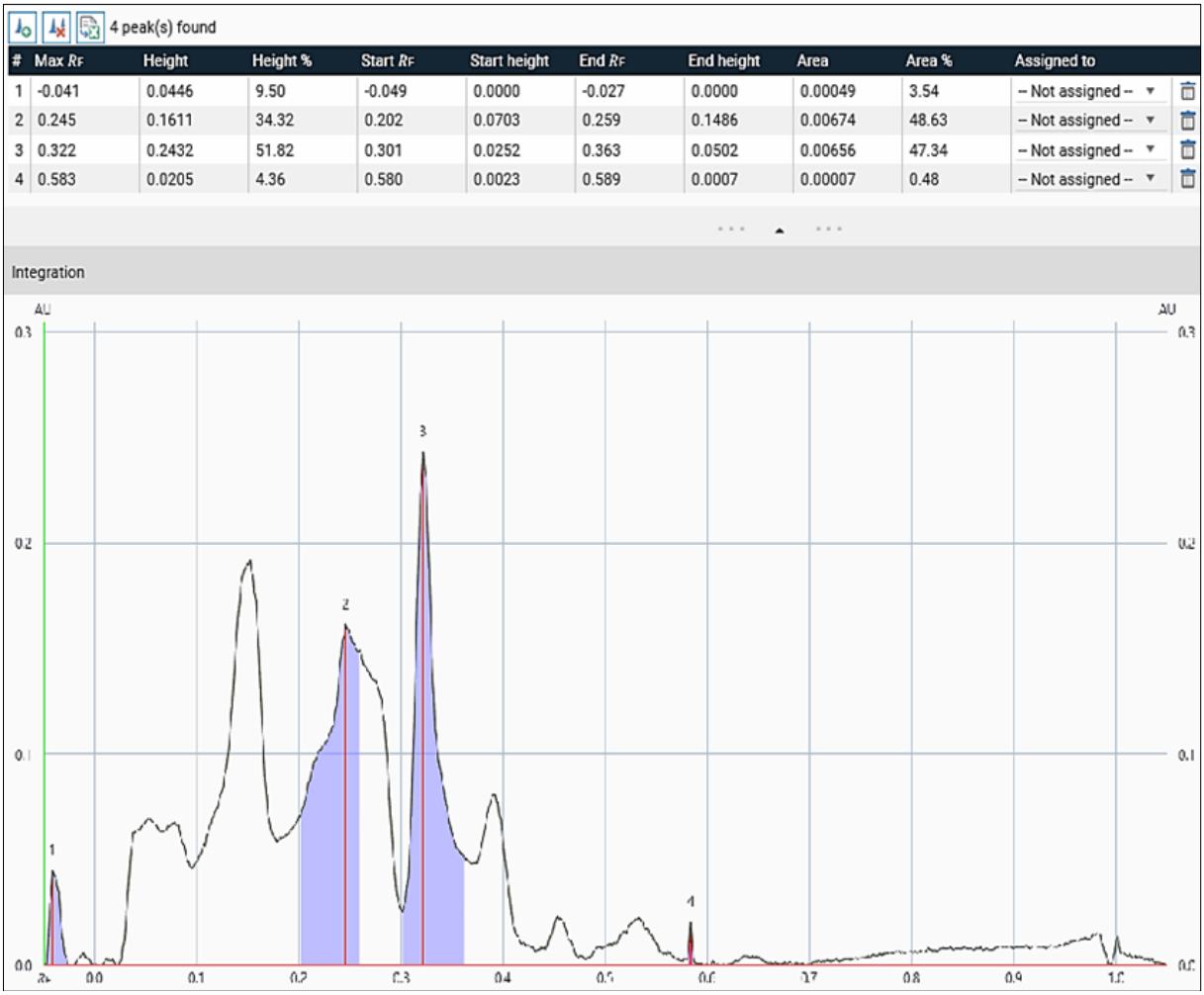


Fig 12: Densitogram of Chloroform extract of Berg-e-Kasondi at 254 nm



#	Max RF	Height	Height %	Start RF	Start height	End RF	End height	Area	Area %	Assigned to
1	-0.041	0.0446	9.50	-0.049	0.0000	-0.027	0.0000	0.00049	3.54	-- Not assigned --
2	0.245	0.1611	34.32	0.202	0.0703	0.259	0.1486	0.00674	48.63	-- Not assigned --
3	0.322	0.2432	51.82	0.301	0.0252	0.363	0.0502	0.00656	47.34	-- Not assigned --
4	0.583	0.0205	4.36	0.580	0.0023	0.589	0.0007	0.00007	0.48	-- Not assigned --

Fig 13

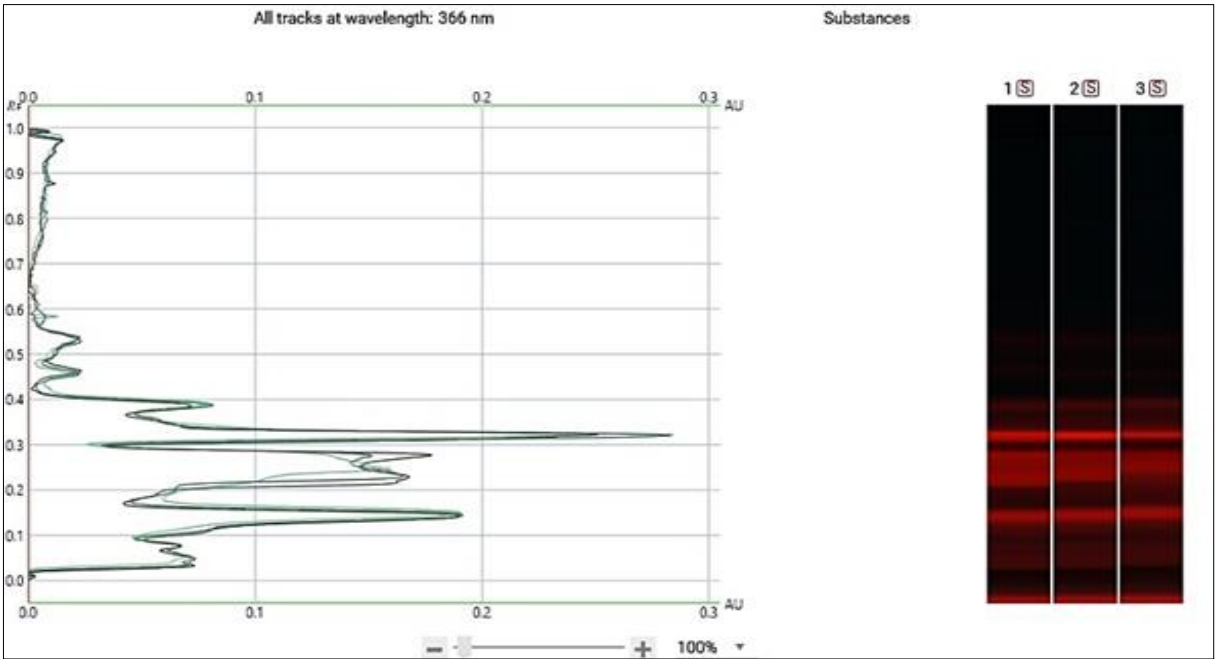


Fig 14: Densitogram of Chloroform extract of Berg-e-Kasondi at 366 nm

7 peak(s) found

#	Max R_F	Height	Height %	Start R_F	Start height	End R_F	End height	Area	Area %	Assigned to
1	0.074	0.3944	19.02	-0.019	0.0190	0.101	0.2134	0.02241	27.79	-- Not assigned --
2	0.120	0.2888	13.93	0.102	0.2134	0.137	0.1967	0.00869	10.78	-- Not assigned --
3	0.193	0.1994	9.62	0.180	0.1808	0.216	0.1599	0.00664	8.23	-- Not assigned --
4	0.307	0.4593	22.16	0.282	0.2480	0.335	0.1934	0.01735	21.51	-- Not assigned --
5	0.398	0.2255	10.88	0.371	0.1113	0.443	0.0369	0.00977	12.11	-- Not assigned --
6	0.835	0.0963	4.65	0.804	0.0389	0.860	0.0397	0.00386	4.78	-- Not assigned --
7	0.991	0.4093	19.74	0.962	0.0839	1.019	0.0010	0.01194	14.80	-- Not assigned --

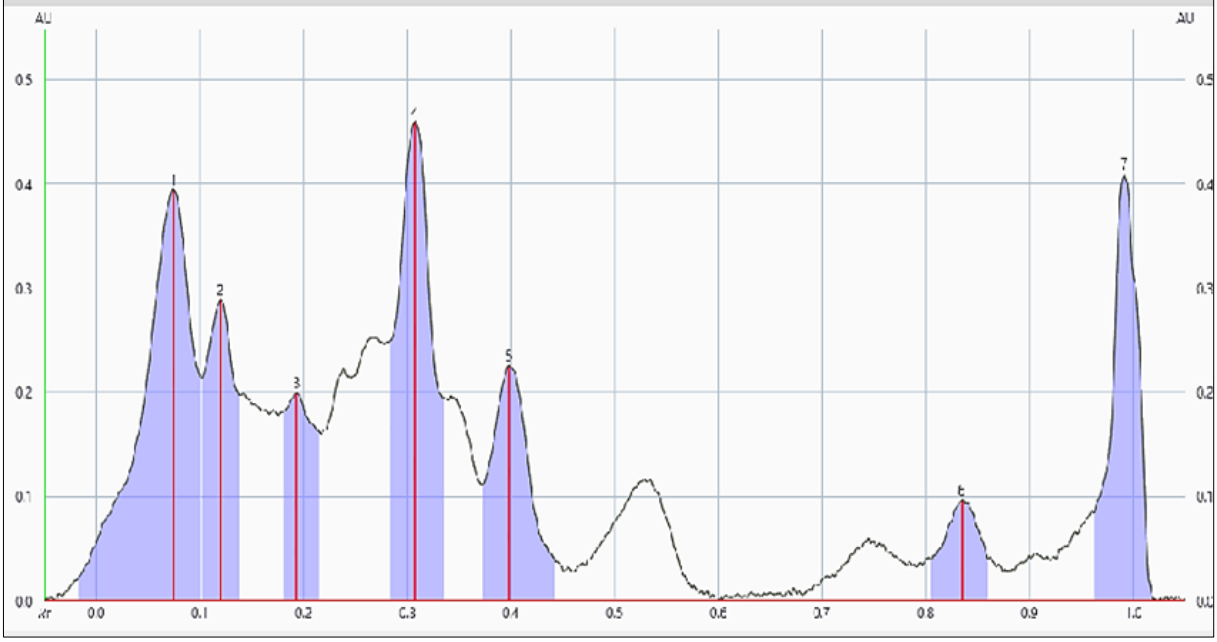


Fig 15

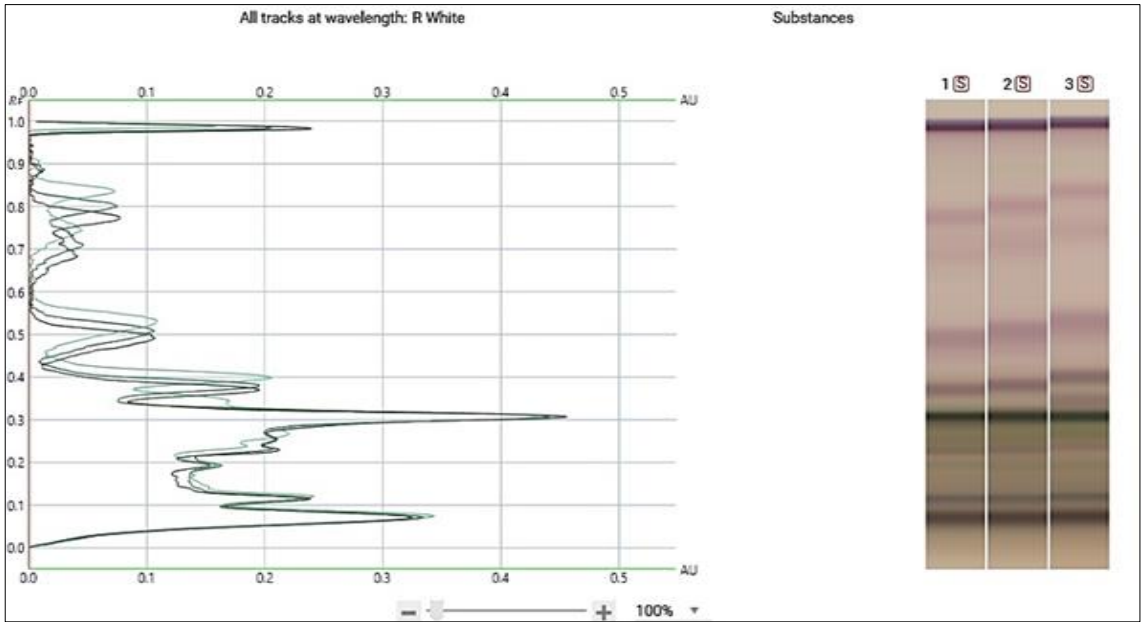


Fig 16: Densitogram of Chloroform extract of Berg-e-Kasondi at 560 nm after derivatization

b) High Performance Thin Layer Chromatography of Alcoholic extract

TLC profile under UV 254nm showed one major peak at Rf value 0.418 and four major peaks at 0.013, 0.278, 0.395 & 0.441 Rf values and under visible region after derivatization with 1% Vanillin-Sulphuric acid showed six spots at Rf values 0.017, 0.149, 0.304, 0.422, 0.765 & 0.971 on TLC plate.

TLC of Alcoholic extract of Berg-e-kasondi

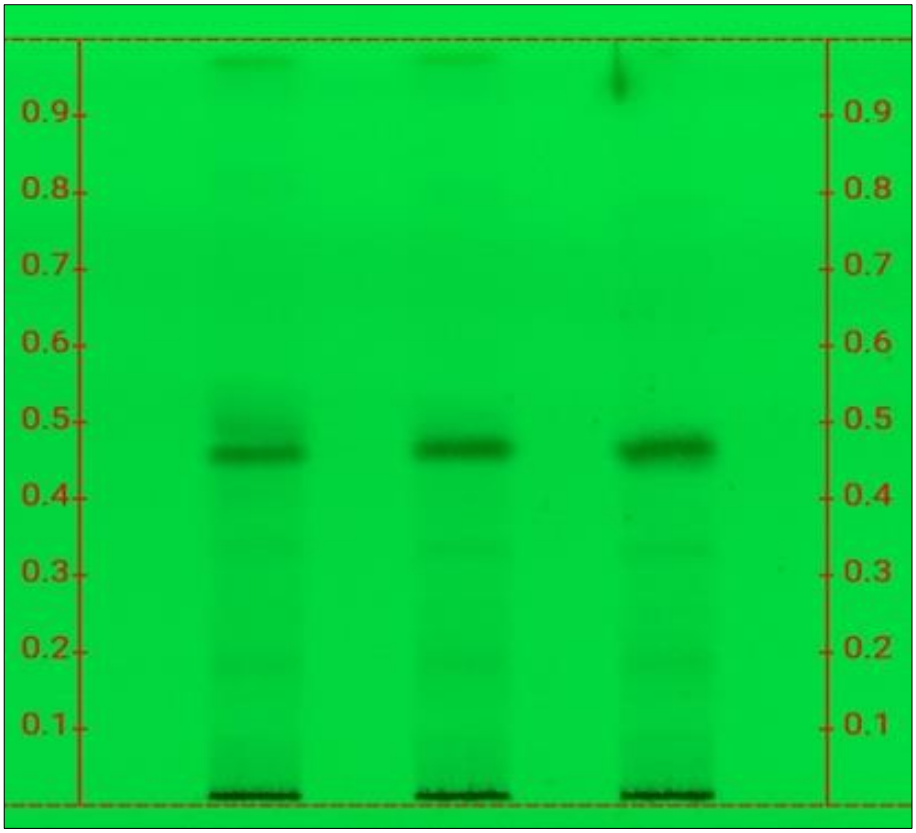


Fig 17: B-I B-II B-III At 254 nm

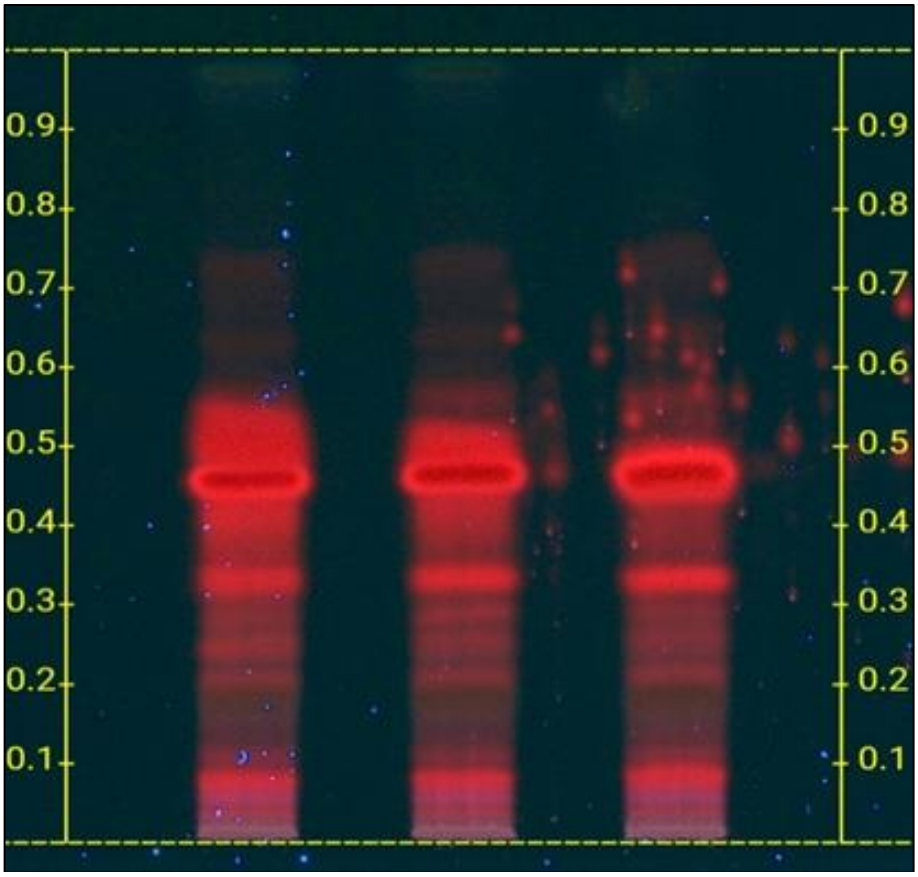


Fig 18: B-I B-II B-III at 366 nm

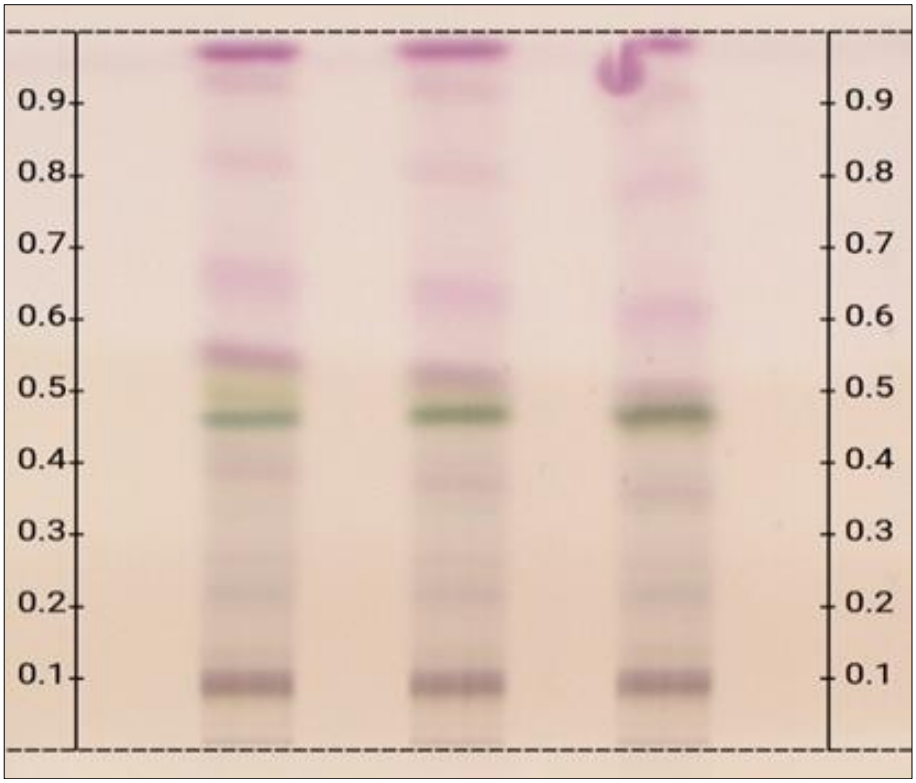
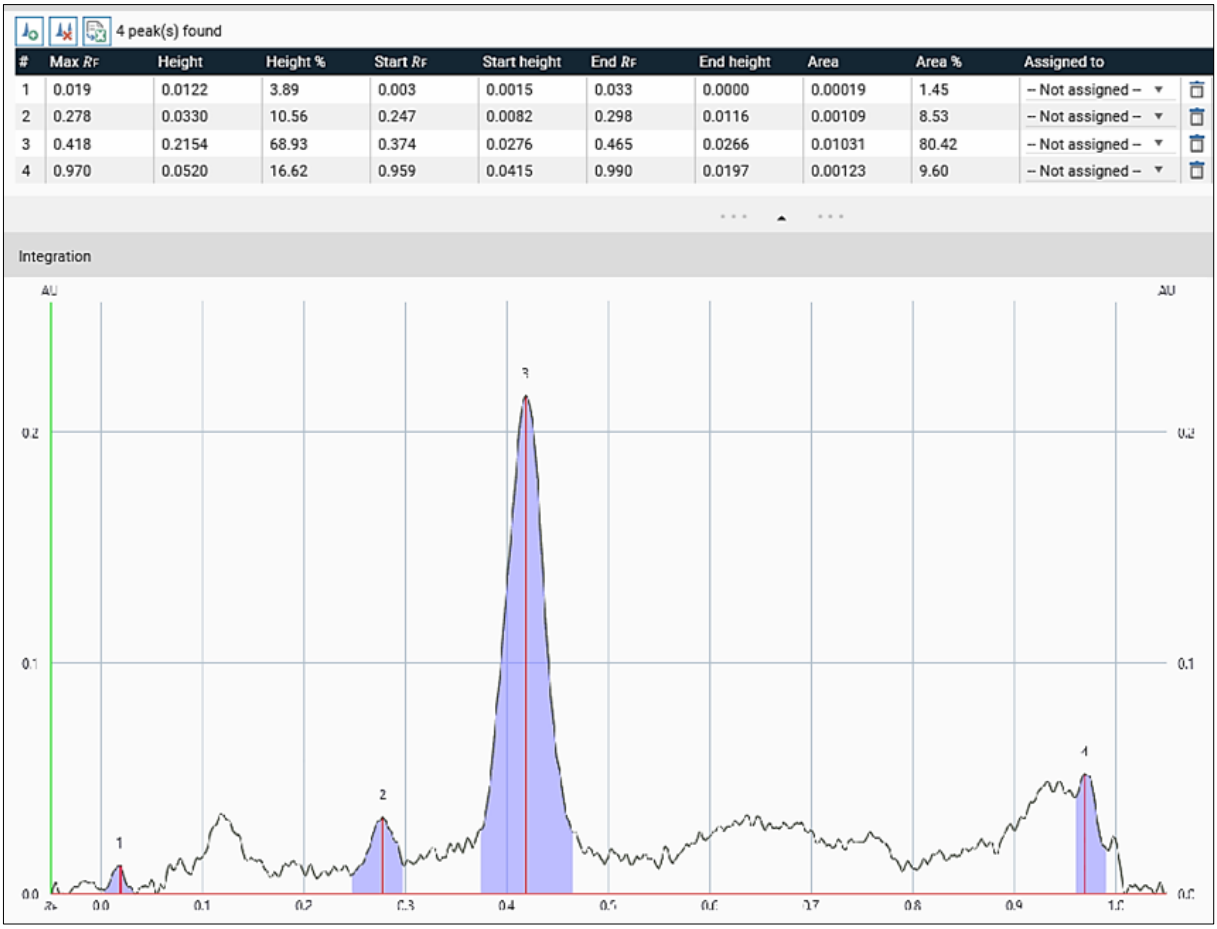


Fig 19: B-I B-II B-III at 560 nm derivatization with VSA



#	Max R_F	Height	Height %	Start R_F	Start height	End R_F	End height	Area	Area %	Assigned to
1	0.019	0.0122	3.89	0.003	0.0015	0.033	0.0000	0.00019	1.45	-- Not assigned --
2	0.278	0.0330	10.56	0.247	0.0082	0.298	0.0116	0.00109	8.53	-- Not assigned --
3	0.418	0.2154	68.93	0.374	0.0276	0.465	0.0266	0.01031	80.42	-- Not assigned --
4	0.970	0.0520	16.62	0.959	0.0415	0.990	0.0197	0.00123	9.60	-- Not assigned --

Fig 20

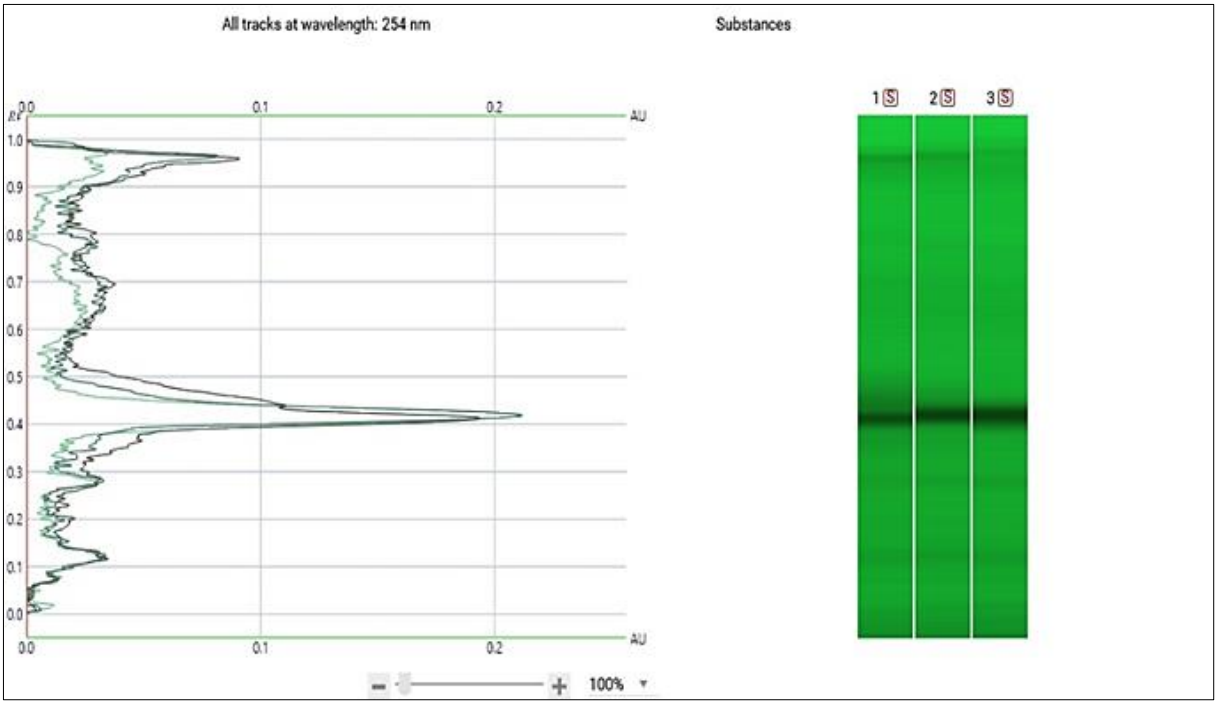


Fig 21: Densitogram of Alcohol extract of Berg-e-Kasondi at 254 nm

10 peak(s) found

#	Max R_F	Height	Height %	Start R_F	Start height	End R_F	End height	Area	Area %	Assigned to
1	0.013	0.1080	11.34	-0.013	0.0578	0.035	0.0178	0.00350	10.57	-- Not assigned --
2	0.150	0.0477	5.00	0.115	0.0000	0.168	0.0278	0.00133	4.01	-- Not assigned --
3	0.196	0.0454	4.77	0.169	0.0274	0.216	0.0341	0.00182	5.49	-- Not assigned --
4	0.232	0.0467	4.91	0.218	0.0339	0.245	0.0351	0.00113	3.41	-- Not assigned --
5	0.278	0.1901	19.96	0.245	0.0351	0.311	0.0321	0.00673	20.33	-- Not assigned --
6	0.395	0.1860	19.53	0.336	0.0466	0.418	0.1133	0.00872	26.36	-- Not assigned --
7	0.441	0.2660	27.92	0.418	0.1133	0.481	0.0213	0.00868	26.22	-- Not assigned --
8	0.578	0.0198	2.08	0.553	0.0052	0.594	0.0077	0.00053	1.61	-- Not assigned --
9	0.611	0.0247	2.60	0.594	0.0077	0.635	0.0037	0.00056	1.70	-- Not assigned --
10	0.765	0.0181	1.90	0.759	0.0000	0.769	0.0009	0.00010	0.30	-- Not assigned --

Integration

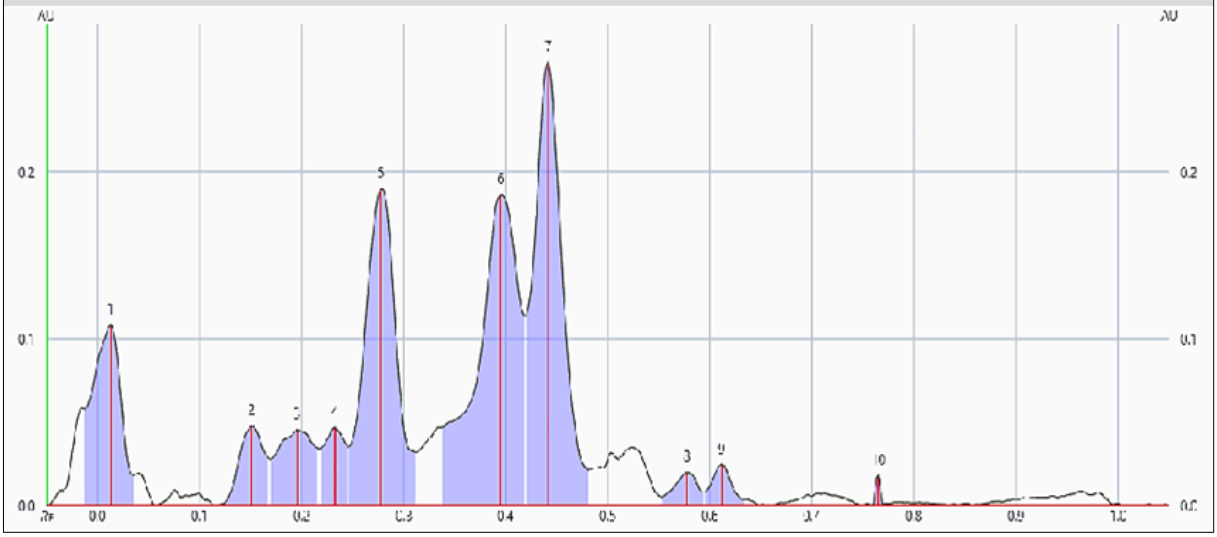


Fig 22

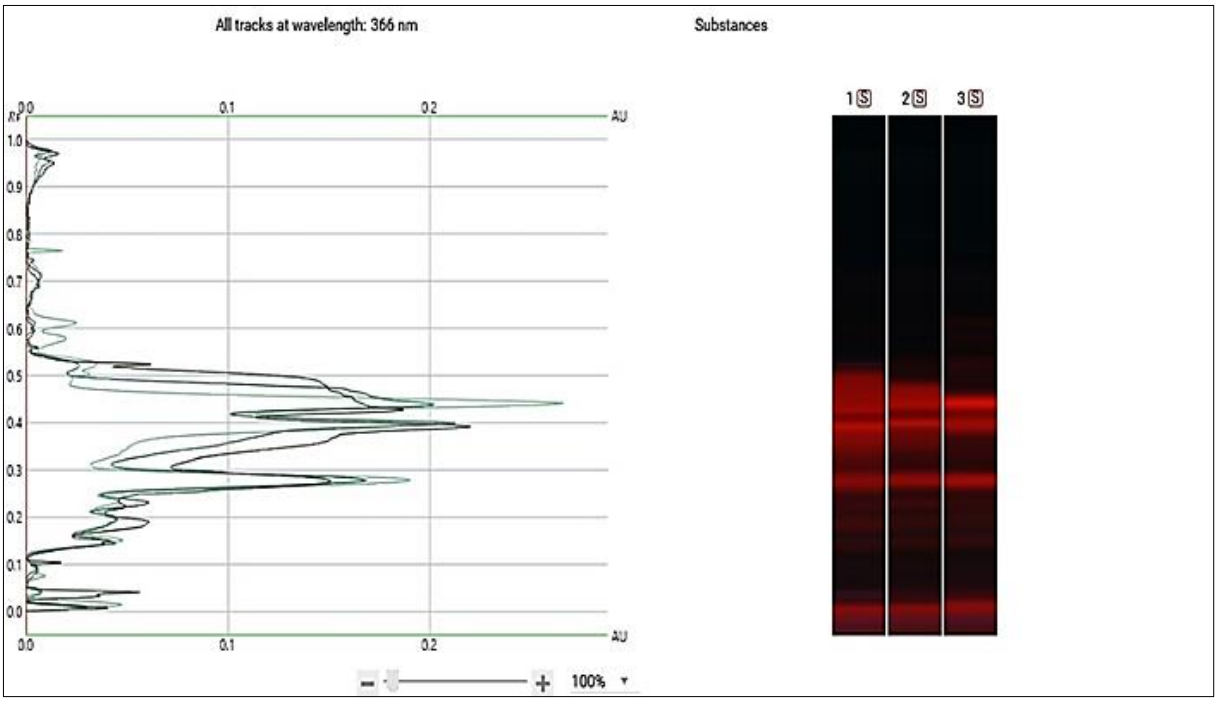
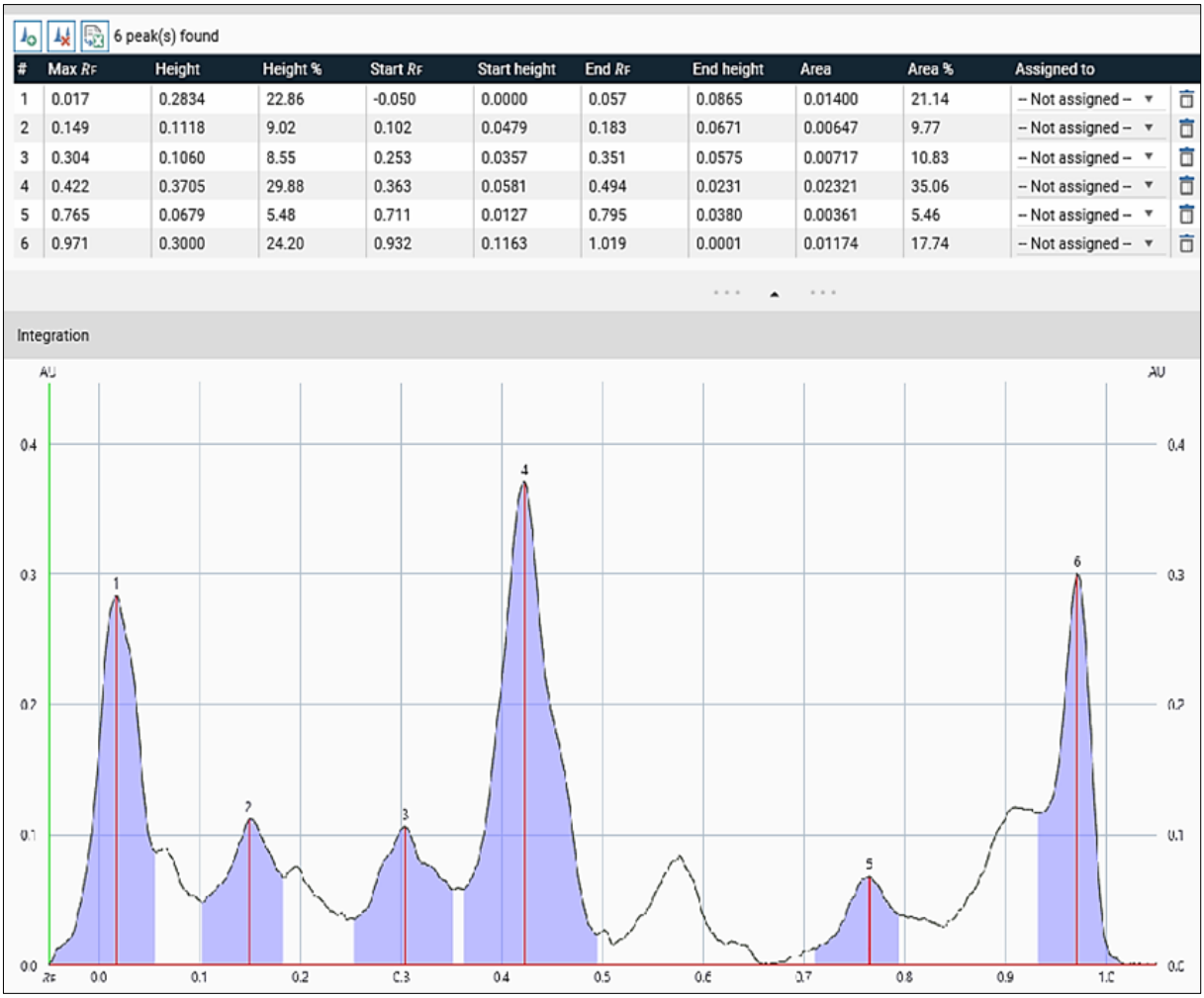


Fig 23: Densitogram of Alcohol extract of Berg-e-Kasondi at 366 nm



#	Max Rf	Height	Height %	Start Rf	Start height	End Rf	End height	Area	Area %	Assigned to
1	0.017	0.2834	22.86	-0.050	0.0000	0.057	0.0865	0.01400	21.14	– Not assigned –
2	0.149	0.1118	9.02	0.102	0.0479	0.183	0.0671	0.00647	9.77	– Not assigned –
3	0.304	0.1060	8.55	0.253	0.0357	0.351	0.0575	0.00717	10.83	– Not assigned –
4	0.422	0.3705	29.88	0.363	0.0581	0.494	0.0231	0.02321	35.06	– Not assigned –
5	0.765	0.0679	5.48	0.711	0.0127	0.795	0.0380	0.00361	5.46	– Not assigned –
6	0.971	0.3000	24.20	0.932	0.1163	1.019	0.0001	0.01174	17.74	– Not assigned –

Fig 24

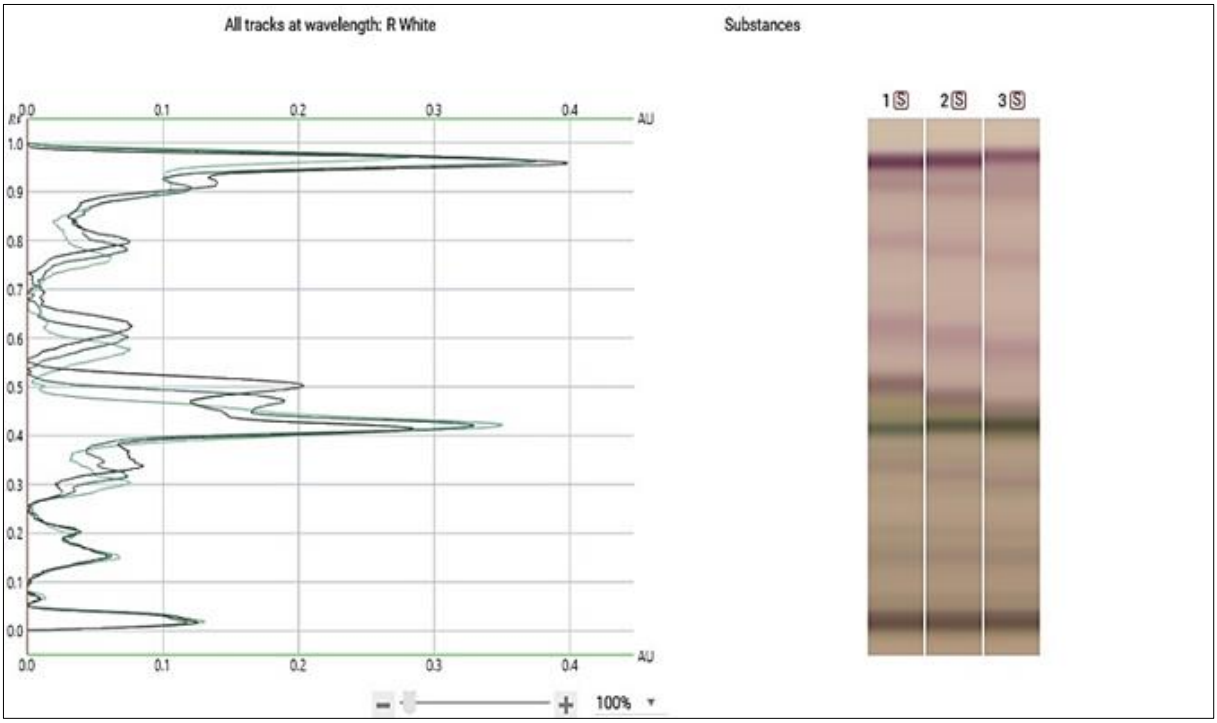


Fig 25: Densitogram of Alcohol extract of Berg-e-Kasondi at 560 nm after derivatization

Discussion and Conclusion

Various parameters like macro and microscopical characteristics, powder studies, physico-chemical analysis chromatographic fingerprinting profile analysis were carried out of Kasondi to established appropriate data. In addition, the obtained information about powder studies and chromatographic profile of them provided supporting referential parameters for identification of the species.

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