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Standardization of polyherbal formulation-Sufoof-e-Ziabetus Dulabi

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Abstract: Standardization of unani herbal formulations is very much essential to justify the quality of a medicine.Unani medicines have played a significant role in maintaining of human health. These medicines are accepted as important therapeutic agents for the treatment of various kind of diseases. But in many instances, it has been noticed that incorrect raw materials have been added in the formulations which has resulted adulterated product in market place. In Unani system of medicine, there are hundreds of single drugs and compound formulations which are used by a large population of several countries to a great advantage. One such drug Sufoof-e-Ziabetus Dulabi is very popular Unani formulation used for Ziabetus Sadiq (Diabetes) and Zof-e- kulya (weakness of kidney). The drug was taken for evaluation through various scientific parameters such as microscopy, physico-chemical analysis and HPTLC fingerprinting. Powder microscopy studies produced in the present study will help in developing pharmacopoeial standards of Sufoof-e-Ziabetus Dulabi

Index Terms **– Compound formulations, physico-chemical analysis, HPTLC finger printing**

Introduction

Tibb-e-Unani (Unani medicine) claims to possess many safe and effective single drugs and compound formulations of herbal, animal and mineral origin which are used to cure a wide range of diseases. Unani compound preparations are commonly used in the four forms viz. solid (Habb, Qurs, Sufoof, Kushta etc.), semi solid (Majoon, Laooq, Marham, Zimaad etc.), liquid (Sheera, Rooh, Sharbat, Tila etc.) and gaseous (Bakhoor, Inkibaab, Ghalia etc.). Sufoofs are the fine powder form of medicinal preparations made of plants, animals and mineral origin drugs. Sufoof-e-Ziabetus Dulabi is light brown powder preparation with aromatic odour and sweet in taste. Sufoof-e-Ziabetus Dulabi is one of the important Unani formulation listed in the National Formulary of Unani Medicine, Part-1. Sufoof-e-Ziabetus Dulabi is commonly used in unani system of medicine for different kind of ailments. The physician of unani system of medicine prescribes this drug for the treatment of Ziabetus Sadiq (Diabetes) and Zof-e- kulya (weakness of kidney).

This classical unani formulation is prepared using seven ingredients-Post-e-Darakht-e-gulnar (Punica granatum L.), Gul-e-Farsi (Punica granatum L.), Dana Anar shireen (Punica granatum L.), Magz-e-Tukhm–e-Anba (Mangifera indica L.), Aamla (Phyllanthus emblica L.), Kishneez Khushk (Coriendrum sativum L.) and Gil-e-Armani (a type of Soil used in many unani preparations).[1, 2, 3,4,5]

The present paper describes the salient features of powder microscopy, physicochemical studies like ash value, extractive value, and pH value and HPTLC studies such as Rf values, Densitogram and HPTLC fingerprint profile in alcoholic and chloroform extract. Similar studies have been carried out and published on unani compound formulation Itrifal Shahatra and Jawarish –e-ood shireen.[10, 11]

2. MATERIAL AND METHODS[8,9,12,13]

The preparation of drug includes identification and authentication, removal of adulterants if any, powdering to required sieve size, method of preparation, ash determination, extractable matter determination, storage, maintenance, testing, preparation of reagents, standardization, etc.

2.1 Ingredients authentication: The raw ingredients were identified by the botanist using pharmacognostical method.

2.2 Drug formulation: Sufoof-e-Ziabetus Dulabi was prepared as per the formulation composition given in National formulary of Unani medicine Part-1.

Table-1: Formulation Composition

S.No.	Crude Drug	Botanical /Mineral Name [6]	Part Used
1.	Post-e-andrun-e- darakht-e-gulnar	*Punica granatum* L.	Stem bark
2.	Gul-e-farsi	*Punica granatum* L.	Flower
3.	Dana anar shireen	*Punica granatum* L.	Seeds
4.	Maghz-e-Tukhm e Anba	*Mangifera indica* L.	Seed
5.	Amla	*Phyllanthus emblica* L.	Fruit
6.	Kishneez Khushk	*Coriendrum sativum* L.	Fruit
7.	Gil-e-Armani	Clay -	Soil

2.3) Powder Microscopy: Take 10-15 grams of compound drug and stir carefully with hot water in a beaker; discard the residue than repeat the process; take the small amount of sediment in a slide and mount with glycerine; take a small amount of residue than treat separately with chloral hydrate than wash with distilled water and mount with glycerine and observe the following characters under microscope.

2.4) Physicochemical analysis: The physico-chemical methods viz., moisture content, ash value, solubility in different solvents, pH values etc. were 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.[7]

2.5) Thin Layer Chromatography [12, 14]

a.)*Preparation of extracts of the sample drug*: The drug sample of 2g was extracted with 40 ml of alcohol and chloroform separately in boiling tubes by ultrasonic sonicator for 30 min at 60^0C. 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 10mm band on Pre-coated Aluminium Sheets of Silica Gel 60 F_{254} (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 at 366nm & 254nm and also by derivatization with 1% Vannillin-sulphuric acid and heated at 105^0C for 5 minutes 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 F_{254} (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.

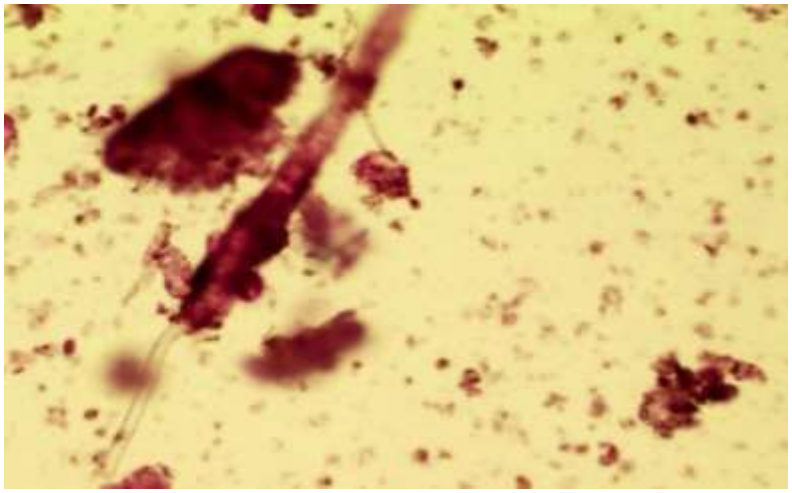
3.RESULT AND DISCUSSION

3.1. Organoleptic Characters: Sufoof-e-Ziabetus Dulabi is a reddish brown powder with aromatic odor and slightly sweet in taste.

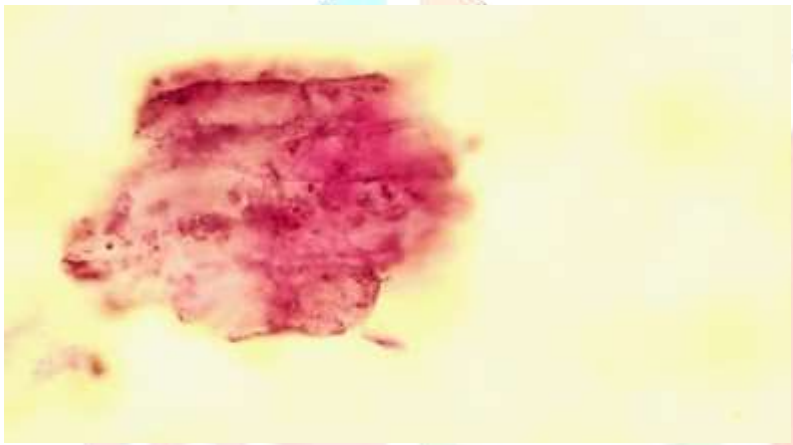
3.2). Microscopical Observation : (Powder Microscopy)

Observe the following characters under microscope fibers with narrow lumen and group of stone cells (**Post-e-Darakt-e-Gulnar**); simple and compound type of starch grains, thin walled parenchyma cells filled with starch grains, **(Magz-e-Tukhm-e-Anba)**, parquetry cells and cells of pericarp (**Kishneez Khusk**), rhomboidal prismatic crystals (**Amla)**, round shaped pollens with smooth exine and intine (**Gul-e-Gulnar**), stone cells (**Anaradana**)

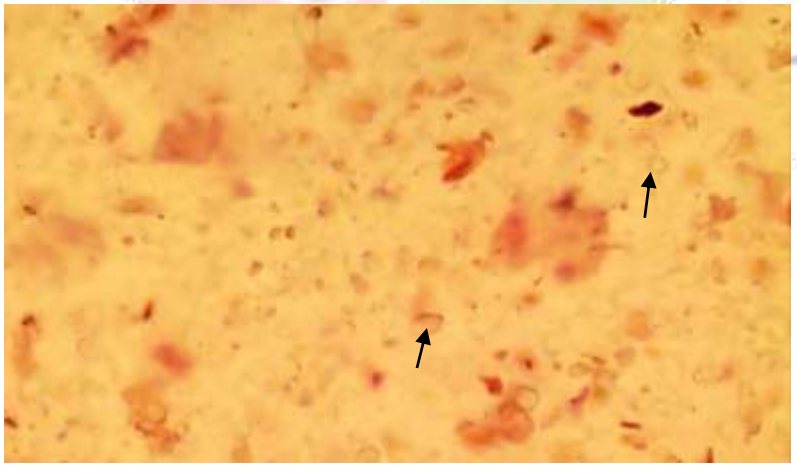
Fig-1(A-I): Powder Microscopy



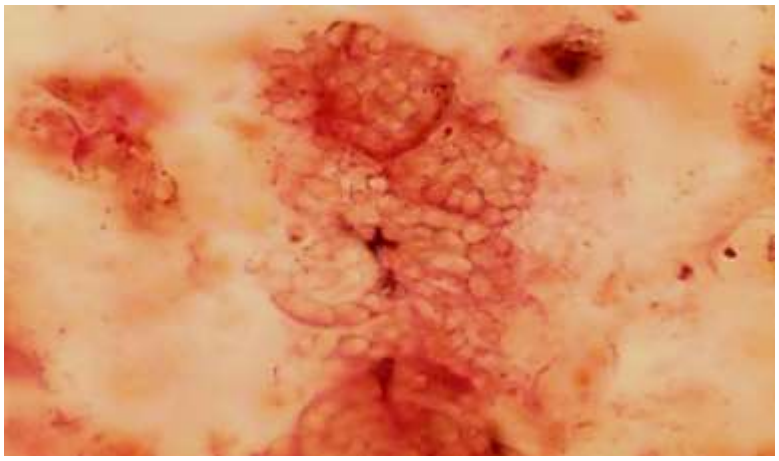
Sufoof e ziabetus fibre of post e darakht- e- gulnar 10x **A**



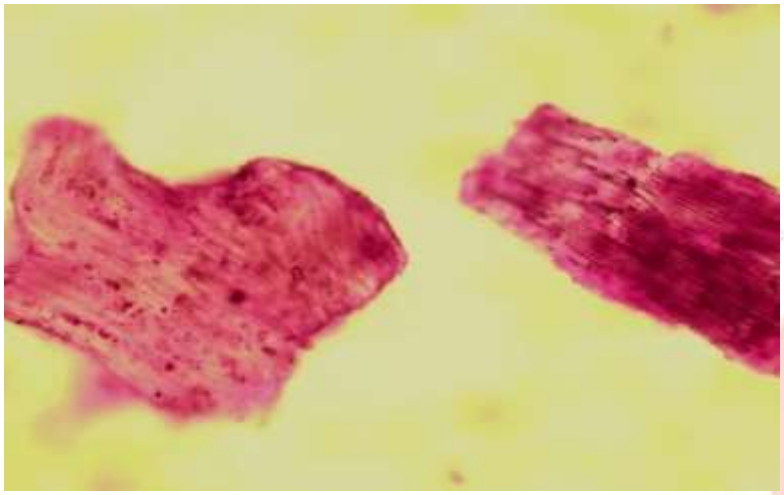
Sufoof e ziabetus group of stone cells of post- e-darakht e gulnar 40x **B**



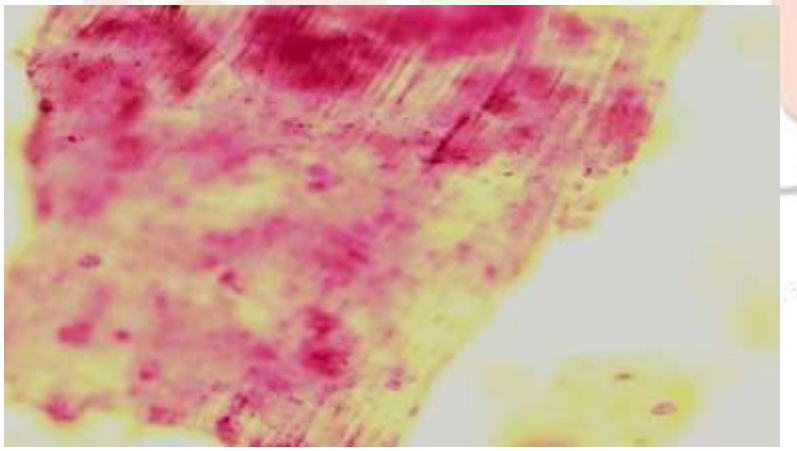
Sufoof e ziabetus simple and compound type starch grains of magz-e-tukhm-e-Anba 40x **C**



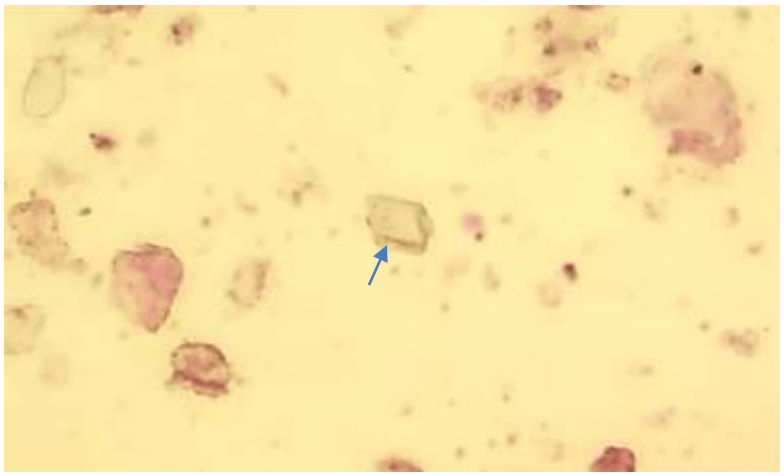
Sufoof e ziabetus parenchyma cells filled with starch grains of magz-e-tukhm-e-Anba 20x **D**



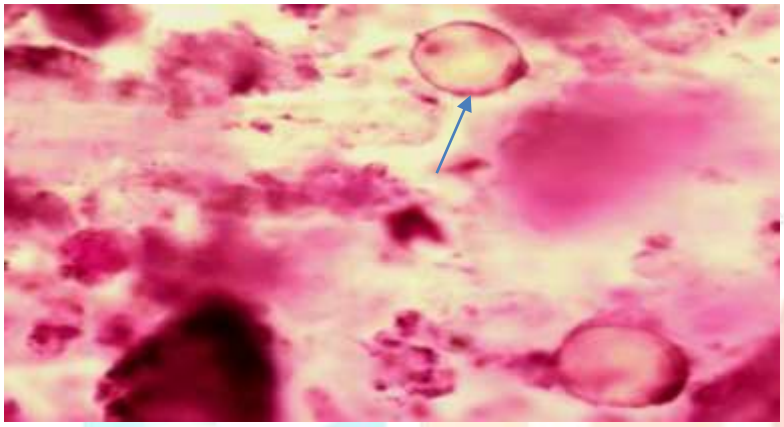
Sufoof e ziabetus cells of pericarp of kishneez khushk 20x **E**



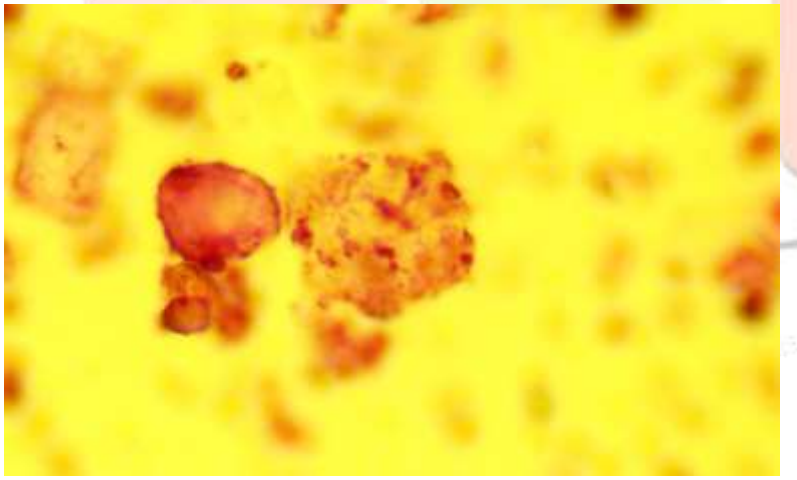
Sufoof e ziabetus parquetry cells of kishneez khushk 40x **F**



Sufoof e ziabetus prismatic crystals of amla 40x **G**



Sufoof e ziabetus pollen grains of gul-e-gulnar 20x **H**



Sufoof e ziabetus stone cells of anardana 20x **I**

3.3). PHYSICO-CHEMICAL ANALYSIS

The Physico-chemical parameters of the formulations Sufoof-e-ziabetus dulabi were studied such as total ash, acid insoluble ash, solubility in water and alcohol, loss in weight on drying at 105 ^{0}C, and pH of 1% & 10% aqueous solution, the results are tabulated in table below.

Table: 2. PHYSICO-CHEMICAL ANALYSIS

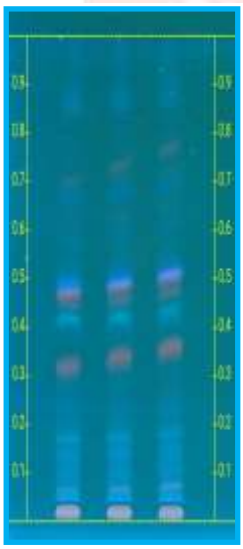
S.No	Parameter	Results
1.	Total ash (*%w/w*)	11.5% - 12.3%
2.	Acid insoluble ash (*%w/w*)	6.4% - 6.8%
3.	Alcohol soluble matter (*%w/w*)	16.50%-17.3%
4.	Water soluble matter (*%w/w*)	31.4%-32.2%
5.	Loss in wt. on drying at 105 ^{0}C (*%w/w*)	5.3%-5.8%
6.	pH of 1% aqueous solution	4.18- 4.30
7.	pH of 10% aqueous solution	3.55-3.65

3.4). HPTLC Profile:

a) High Performance Thin Layer Chromatography of Alcoholic extract:

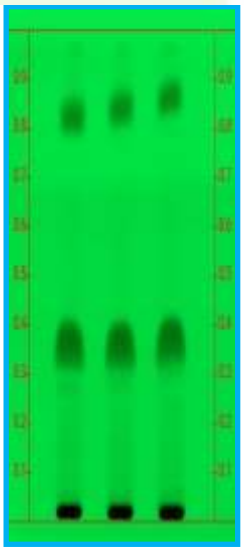
TLC profile under UV 366nm showed five major peaks at R_f values 0.401, 0.51, 0.68, 0.88 & 0.96 and six minor peaks at various R_f values and under UV 254nm showed five major peaks at R_f values 0.32, 0.42, 0.64, 0.80, & 0.91 and six minor peaks at various R_f values and under visible region after derivatization with 1% Vannillin-Sulphuric acid showed six spots at R_f values 0.03, 0.11, 0.19, 0.40, 0.80, and 0.98 on TLC plate. (Fig 2.A-C)

Fig. 2. (A-C) HPTLC of Alcoholic extract of Sufoof-e-Ziabetus Dulabi Track 1: Sample-I; Track 2: Sample-II; Track 3: Sample-III



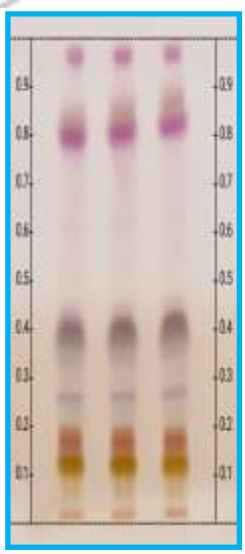
UV 366nm

A



UV 254nm

B



Derivatization with VSA

C

Fig. 3 HPTLC Fingerprint Profile of alcoholic extract of Sufoof-e-Ziabetus Dulabi at UV 366nm

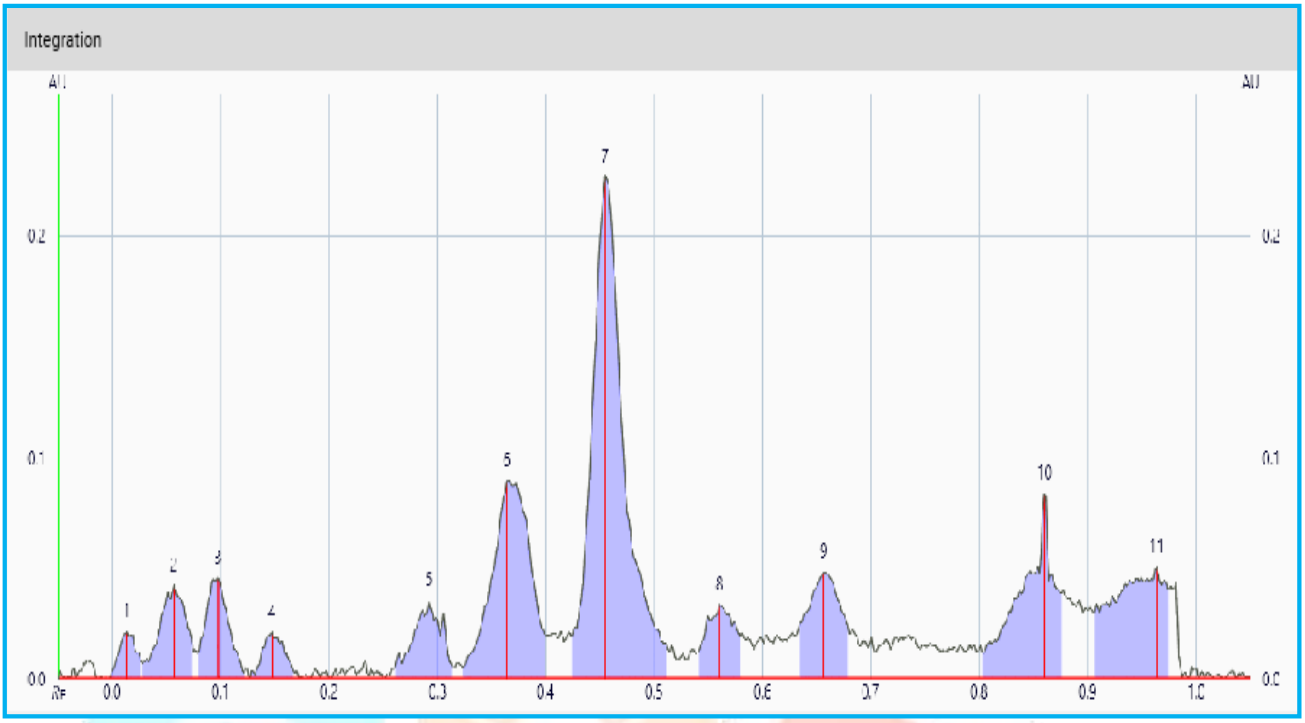


Table 3: Peak list of alcoholic extract of Sufoof-e-Ziabetus Dulabi at UV 366nm

Peak no	Area	Area %	Height	R_f value
1	0.00039	1.62	0.0068	0.02
2	0.00117	4.91	0.0124	0.07
3	0.00111	4.65	0.0020	0.12
4	0.00048	2.01	0.0022	0.17
5	0.00105	4.40	0.0050	0.31
6	0.00370	15.56	0.0194	0.40
7	0.00777	32.67	0.0124	0.51
8	0.00099	4.16	0.0193	0.58
9	0.00166	6.98	0.0203	0.68
10	0.00272	11.42	0.0386	0.88
11	0.00276	11.61	0.0399	0.97

Fig-4. Densitogram of alcoholic extract of Sufoof-e-Ziabetus Dulabi at UV 366nm

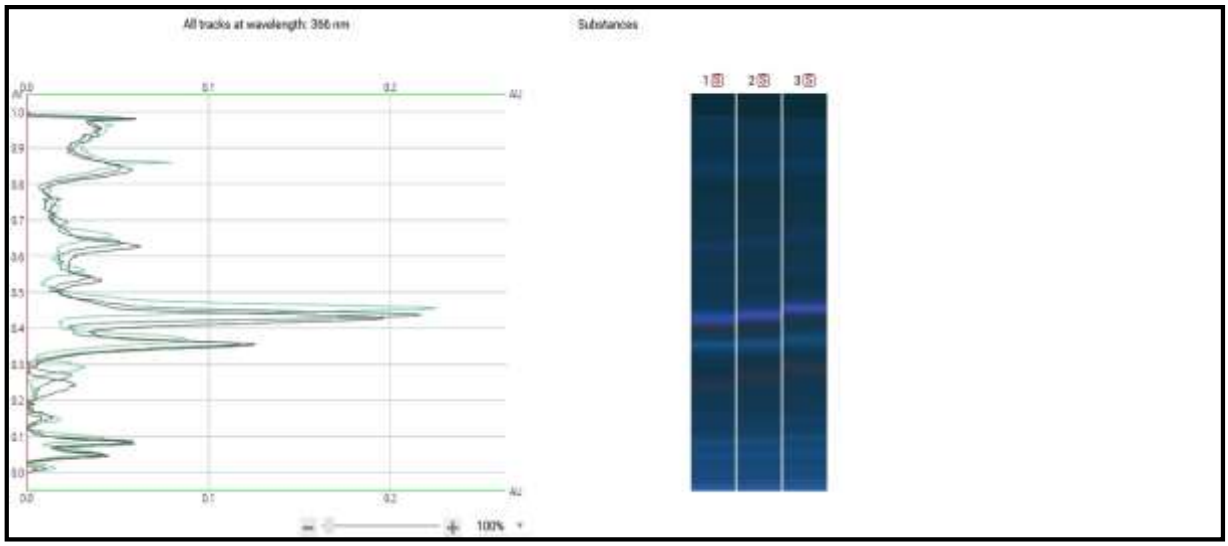


Fig-5 HPTLC Fingerprint Profile of alcoholic extract of Sufoof-e-Ziabetus Dulabi at UV 254nm

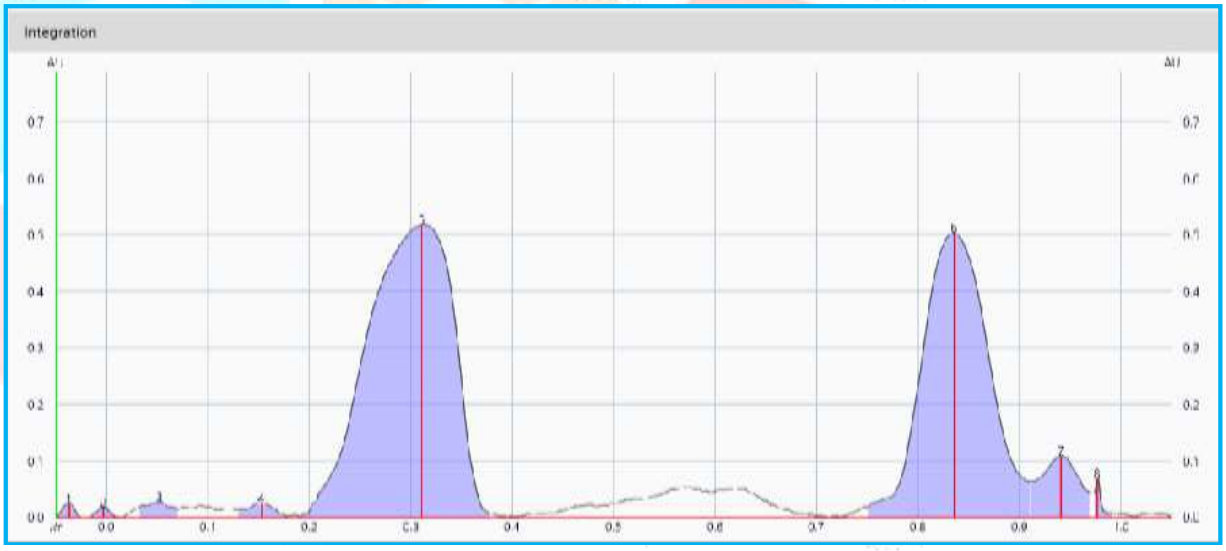


Table 4: Peak list of alcoholic extract of Sufoof-e-Ziabetus Dulabi at UV 254nm

Peak no	Area	Area %	Height	R_f value
1	0.00033	0.34	0.0000	0.00
2	0.00025	0.26	0.0001	0.01
3	0.00085	0.87	0.0147	0.07
4	0.00079	0.82	0.0023	0.18
5	0.05138	52.88	0.0071	0.38
6	0.03821	39.32	0.0637	0.91
7	0.00483	4.97	0.0439	0.97
8	0.00052	0.53	0.0077	0.99

Fig-6 Densitogram of alcoholic extract of Sufoof-e-Ziabetus Dulabi at UV 254nm

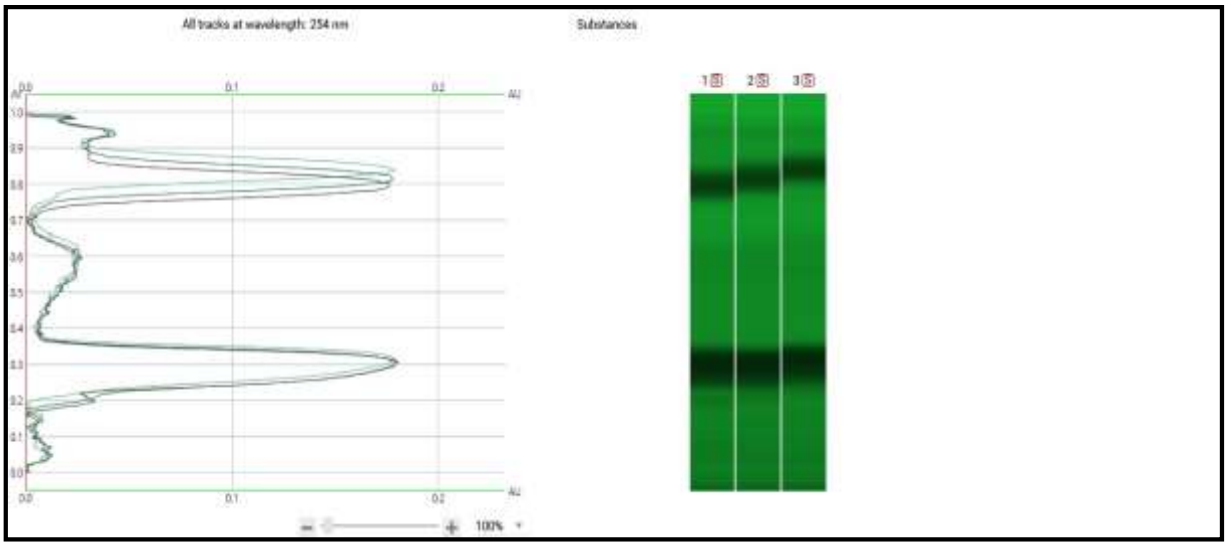


Fig-7.HPTLC Fingerprint profile of alcoholic extract of Sufoof-e-Ziabetus Dulabi after derivatization with VS reagent at 560nm

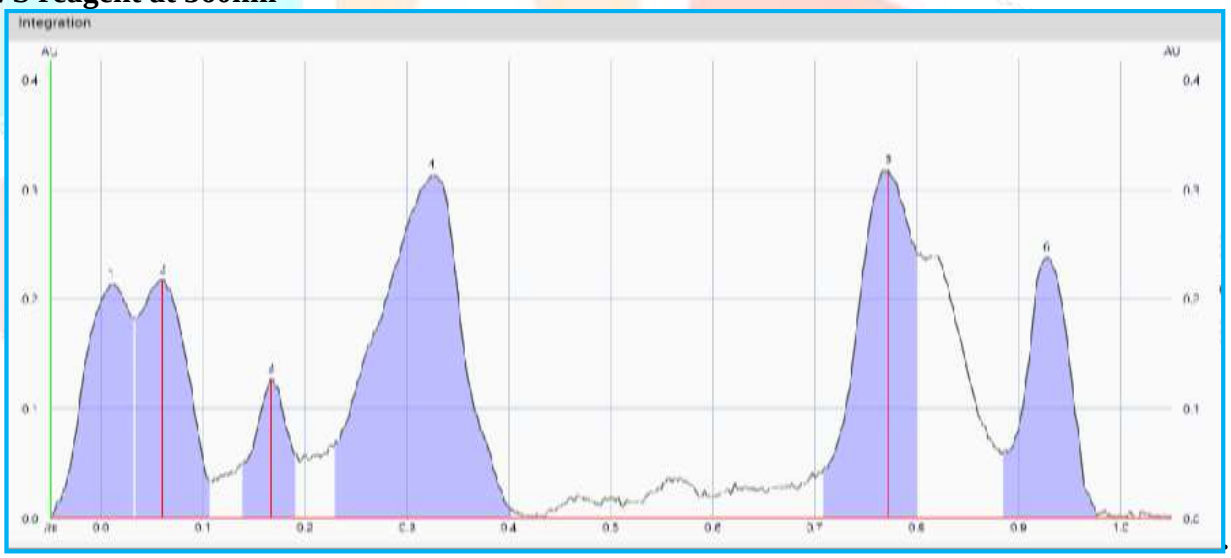
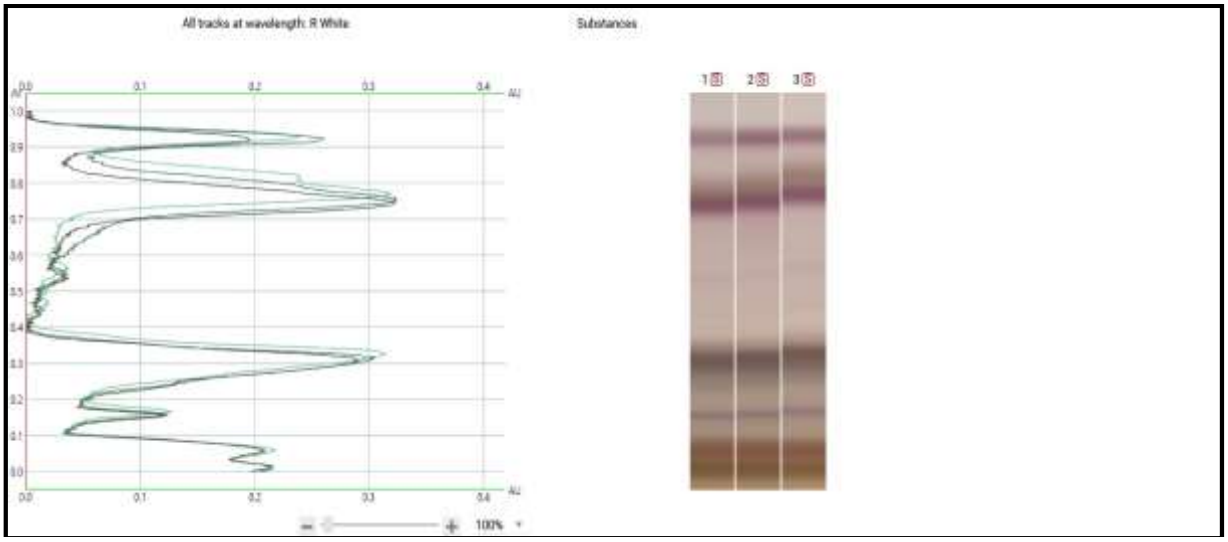


Table 5: Peak list of alcoholic extract of Sufoof-e-Ziabetus Dulabi after derivatization with VS reagent at 560nm.

Peak no	Area	Area %	Height	R_f value
1	0.01095	12.38	0.1819	0.03
2	0.01194	13.51	0.0320	0.11
3	0.00455	5.15	0.0588	0.19
4	0.01986	33.78	0.0072	0.40
5	0.01922	21.74	0.2404	0.80
6	0.01189	13.45	0.0027	0.98

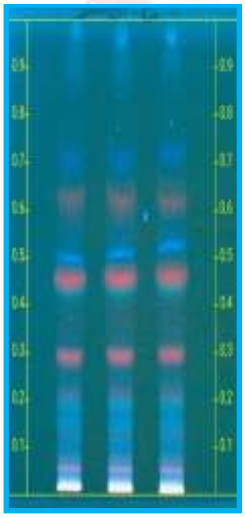
Fig-8.Densitogram of alcoholic extract of Sufoof-e-Ziabetus Dulabi after derivatization with VS reagent at 560nm



b) High Performance Thin Layer Chromatography of Chloroform extract:

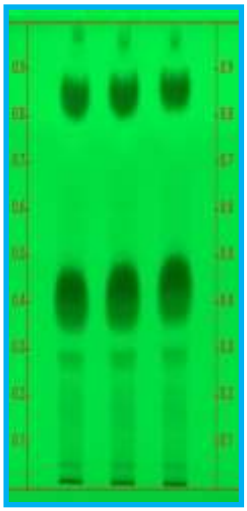
TLC profile under UV 366nm showed two peaks at R_f values 0.11 & 0.99 and under UV 254nm showed eight peaks at R_f values 0.03, 0.11, 0.15, 0.19, 0.29, 0.61, 0.76, & 1.01 and under visible region after derivatized with 1% Vannillin-Sulphuric acid showed eight peaks at R_f values 0.01, 0.12, 0.21, 0.27, 0.36, 0.61, 0.78 & 1.01 on TLC plate.

Fig. 9 (A-C). TLC of Chloroform extract of Sufoof-e-Ziabetus Dulabi Track 1: Sample-I; Track 2: Sample-II; Track 3: Sample-III



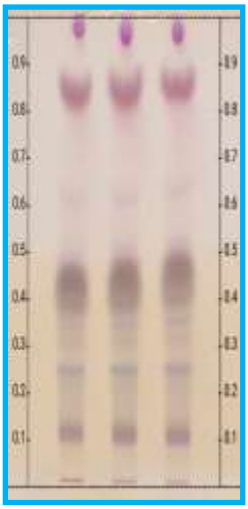
UV 366nm

A



UV 254nm

B



Derivatization with VSA

C

Fig. 10.HPTLC Fingerprint Profile of Chloroform extract of Sufoof-e-Ziabetus Dulabi at UV 366nm

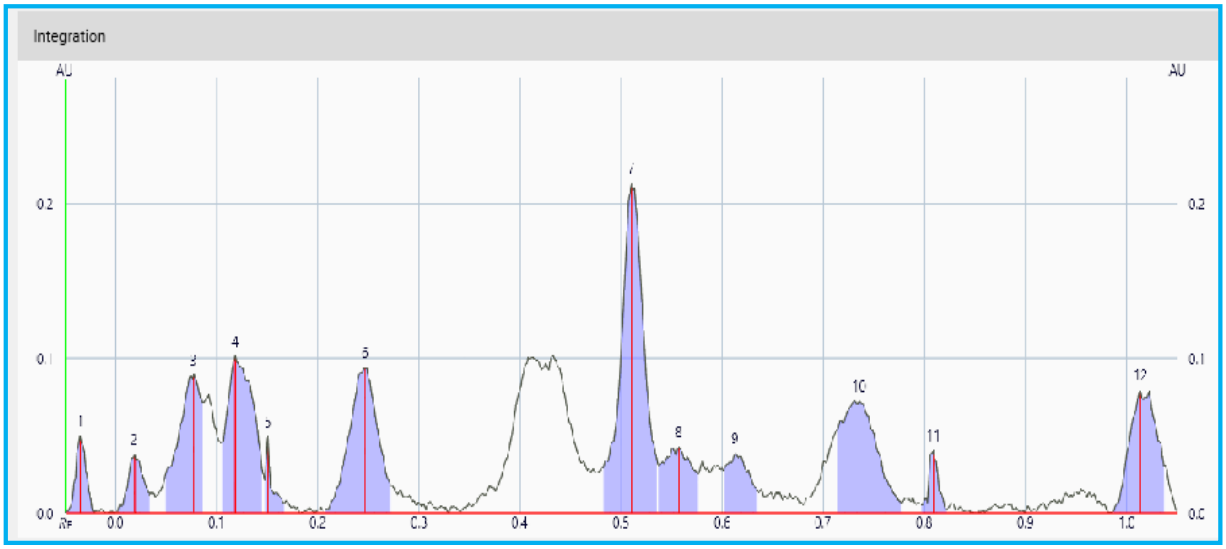


Table 6: Peak list of Chloroform extract of Sufoof-e-Ziabetus Dulabi at UV 366nm

Peak no	Area	Area %	Height	R_f value
1	0.00059	2.46	0.0000	0.00
2	0.00067	2.78	0.0122	0.03
3	0.00230	9.56	0.0716	0.09
4	0.00310	12.90	0.0247	0.15
5	0.00035	1.45	0.0058	0.17
6	0.00308	12.83	0.0176	0.27
7	0.00567	23.61	0.0306	0.54
8	0.00138	5.73	0.0251	0.58
9	0.00101	4.22	0.0135	0.64
10	0.00297	12.35	0.0070	0.78
11	0.00046	1.92	0.0020	0.82
12	0.00245	10.20	0.0302	1.04

Fig. 11. Densitogram of Chloroform extract of Sufoof-e-Ziabetus Dulabi at UV 366nm

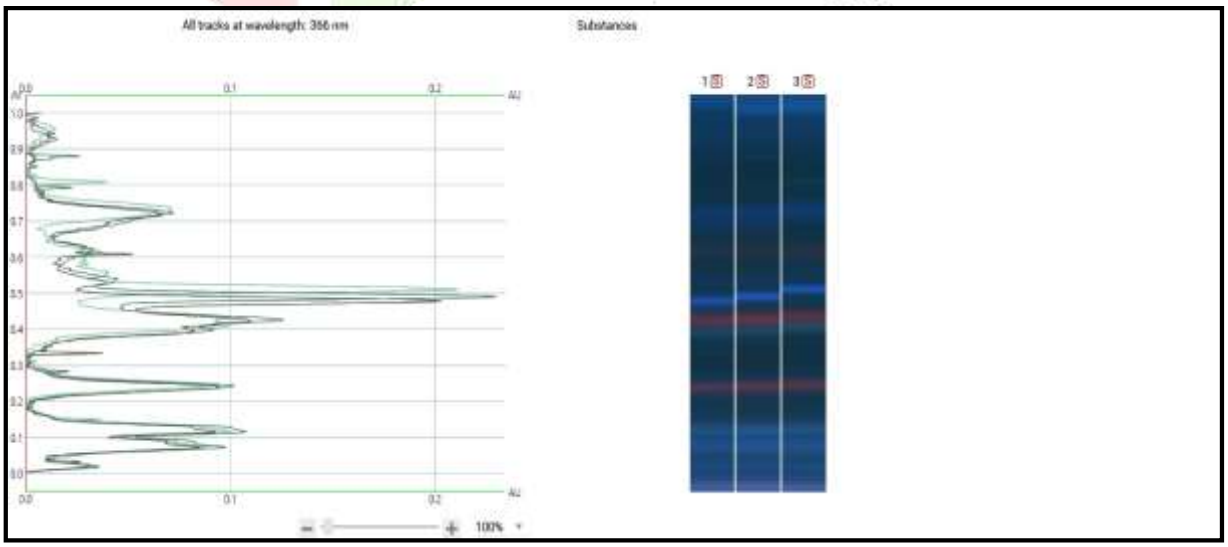


Fig. 12. HPTLC Fingerprint profile of Chloroform extract of Sufoof-e-Ziabetus Dulabi at UV 254nm

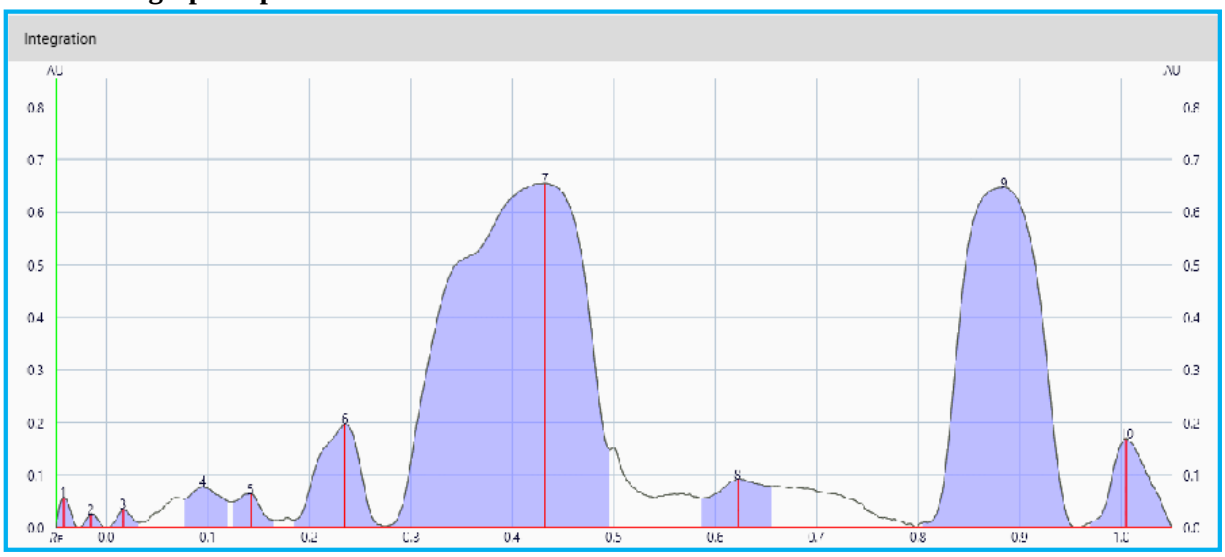


Table 7: Peak list of Chloroform extract of sufoof-e-Ziabetus Dulabi at UV 254nm

Peak no	Area	Area %	Height	R_f value
1	0.00070	0.38	0.0000	0.00
2	0.00034	0.18	0.0000	0.00
3	0.00056	0.31	0.0098	0.03
4	0.00286	1.56	0.0492	0.12
5	0.00180	0.98	0.0129	0.16
6	0.00918	5.02	0.0026	0.27
7	0.09883	54.04	0.1477	0.50
8	0.00539	2.95	0.0779	0.66
9	0.05629	30.78	0.0000	0.95
10	0.00695	3.80	0.0010	1.05

Fig. 13. Densitogram of Chloroform extract of Sufoof-e-Ziabetus Dulabi at UV 254nm

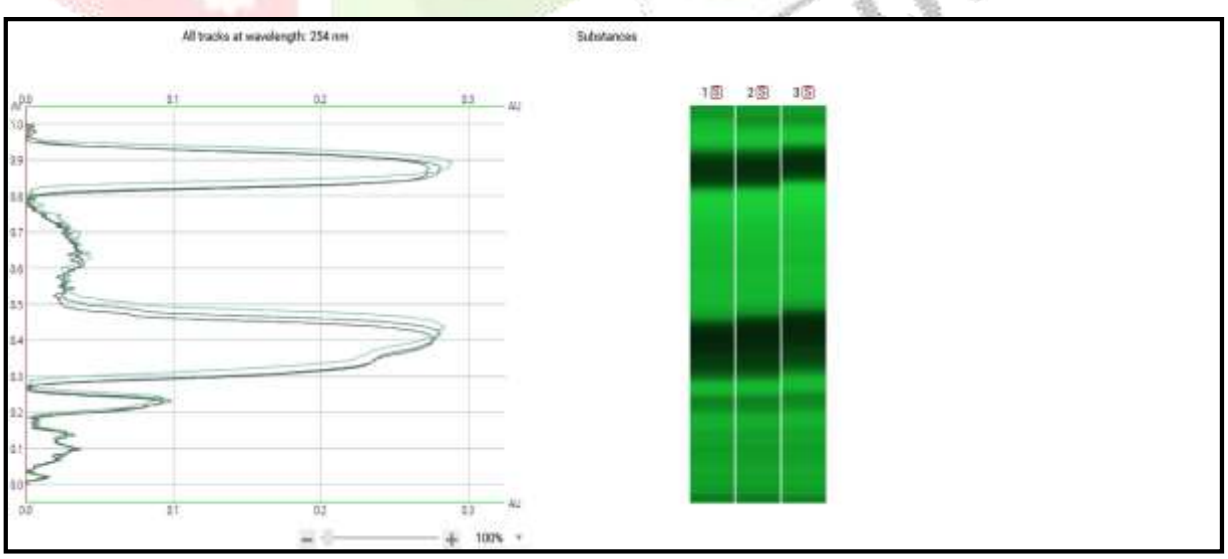


Fig. 14.HPTLC Fingerprint profile of Chloroform extract of Sufoof-e-Ziabetus Dulabi after derivatization with VS reagent at 560nm.

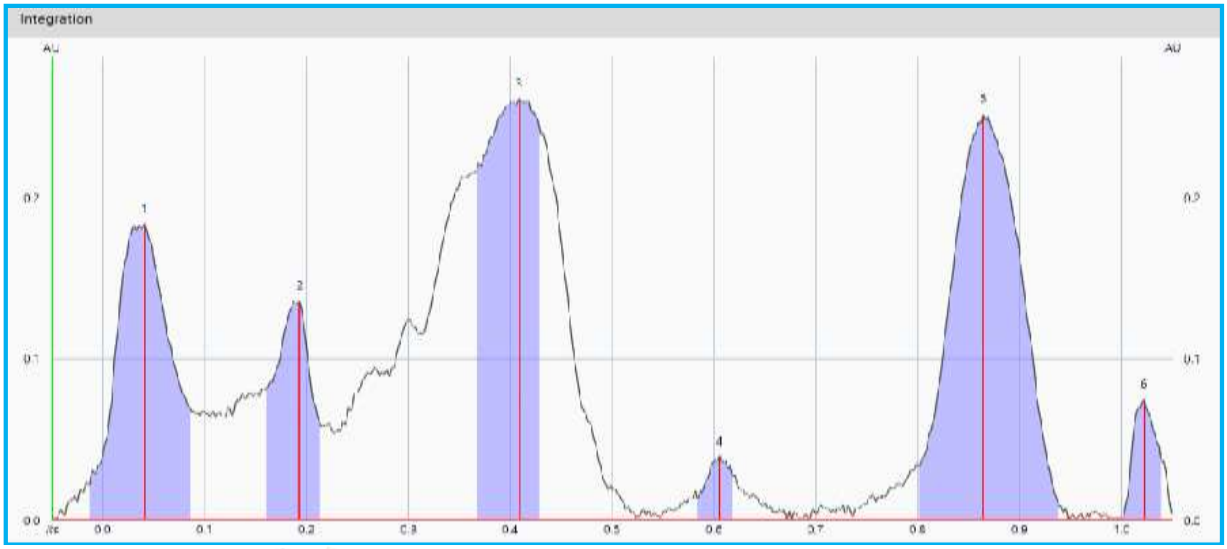
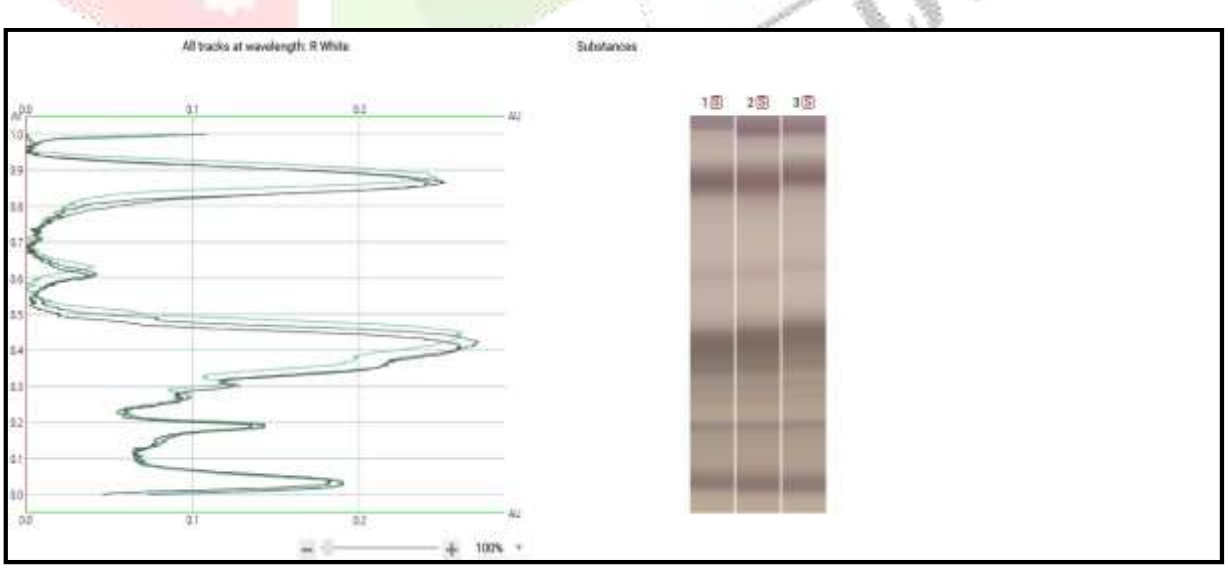


Table 8: Peak list of Chloroform extract of sufoof-e-Ziabetus Dulabi after derivatization with VS reagent at 560nm.

Peak no	Area	Area %	Height	R_f value
1	0.01143	20.54	0.0667	0.09
2	0.00555	9.98	0.0576	0.22
3	0.01586	28.51	0.2389	0.43
4	0.00105	1.89	0.0267	0.62
5	0.01981	35.61	0.0083	0.94
6	0.00193	3.47	0.0357	1.04

Fig. 15. Densitogram of Chloroform extract of Sufoof-e-Ziabetus Dulabi after derivatization with VS at 560nm



Conclusion: The evaluated data such as powder microscopy, physicochemical, HPTLC studies such as R_f values, densitogram and HPTLC fingerprint profile in alcoholic extract and chloroform extract will certainly help in ensuring the quality and purity of the drug.

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