



Standardisation and HPTLC fingerprinting Profile of a Unani Compound formulation Jawarish-Ood-sheerin with modern techniques

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Abstract: The Unani system of medicine prescribes large number of classical herbal formulations to cure the different types of diseases. Jawarish-Ood-Sheerin, a unani herbal formulation is prepared in combination of herbal ingredient like Darchini (*Cinnamoum zeylanicum*), Jauzbuwa (*Myristica fragrans*), Ood hindi (*Aquilaria agallocha*), Zafran (*Crocus sativus*), Heel khurd (*Eletteria cardamomum*), Kulanjan (*Alpinia galangal*), Filfil Daraz (*Piper longum*), Asaroon (*Asarum europium*) and Saleekha (*Cinnamomum cassia*). The Unani physician prescribes the drug Jawarish-ood-sheerin to cure the ailments of Is-hal (Diarrhoea), Zof-e-Hazm (Indigestion) and Zof-e-Ishteha (Anorexia). The formulation was subjected to evaluate the various parameters like powder microscopy, physico-chemical parameters like ash value, extractive value and pH values for 1% and 10% aqueous solutions and TLC/HPTLC finger prints of chloroform and alcohol extracts. The evaluated data will help to lay down pharmacopoeial standards and TLC/HPTLC finger prints for the drug Jawarish-Ood-Sheerin.

Index Terms - Jawarish-Ood-Sheerin, pharmacopoeial standards, physico-chemical, HPTLC

I. INTRODUCTION

II. Jawarish is a semisolid medicinal preparation where one or more single drug of plants, is mixed in powder form. Jawarish-Ood-Sheerin is one of the ancient commonly used classical formulation. It is dark brown semi-solid sticky preparation with aromatic odour and sweet in taste. Jawarish - ood-sheerin is one of the important Unani formulation categorized under the Majooniath categories, listed in the National Formulary of Unani Medicine, Part-1.

III. The drug Jawarish - ood-sheerin is prescribed for the ailments of Is hal (Diarrhoea), Zof-e-Hazm (Indigestion), Zof-e-Ishteha (Anorexia).[1] This polyherbal formulation consists of nine ingredients like Darchini (*Cinnamoum zeylanicum*), Jauzbuwa (*Myristica fragrans*), Ood hindi (*Aquilaria agallocha*), Zafran (*Crocus sativus*), Heel khurd (*Eletteria cardamomum*), Kulanjan (*Alpinia galangal*), Filfil Daraz (*Piper longum*), Asaroon (*Asarum europaeum*), Saleekha (*Cinnamomum cassia*)[2,6].

IV. In order to lay down the pharmacopoeial standards, the drug was prepared in laboratory scale and subjected to microscopical studies and physicochemical studies. The present paper describes the salient features of powder microscopy, physicochemical studies like ash values, extractive values, pH values and TLC/HPTLC finger prints. [3,9]

2. MATERIAL AND METHODS

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. [4,5]

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

2.2 Drug formulation: Jawarish-Ood-Sheerin was prepared as per the formulation composition given in National formulary of Unani medicine Part-1.[1]

Table-1: Formulation Composition

S.No.	Unani name	Botanical name[2]	Part used
1.	Ood Hindi	<i>Aquilaria agallocha</i>	Wood
2.	Darchini	<i>Cinnamomum zeylanicum</i>	Bark
3.	Jauzbuwa	<i>Myristica fragrans</i>	Fruit
4.	Saleekha	<i>Cinnamomum cassia</i>	Bark
5.	Heel khurd	<i>Elettaria cardamomum</i>	Fruit
6.	Filfil Daraz	<i>Piper longum</i>	Fruit
7.	Khulanjan	<i>Alpinia galanga</i>	Rhizome
8.	Asaroon	<i>Asarum europaeum</i>	Root
9.	Zafran	<i>Crocus sativus</i>	Stigma

2.3 Powder Microscopy: The drug sample 10-15 grams was taken with 50 ml of hot water in a beaker and stirred carefully until the sample is completely dispersed in water. The residue obtained was then discarded. A small amount of sediment was taken in a slide and mounted with glycerine; also a small amount of residue was taken and treated separately with chloral hydrate, washed with distilled water and mounted in glycerine and silent features of the drug were observed in different mounts under microscope.

2.4 Physicochemical analysis: The physico-chemical methods viz., ash values, solubility in different solvents, pH values etc. were useful tools in standardization of a herbal product for maintaining the consistency of the drug. The drug samples were subjected to the standardization of physicochemical parameters and analysed as per the standard method. [7]

2.5 HPTLC Finger printing [8,10]

a. Preparation of extracts of the drug:

The Semisolid drug sample of Jawarish-Ood-Sheerin used for leaching out sugar from it, the dried drug sample of 2gm was added to the 40 ml of alcohol and chloroform separately in boiling tubes and then it was heated at 60°C by ultrasonic cleaner for 30 minutes. The extracts of samples were filtered and concentrated to 5 ml. Then the concentrated extracts were used to carry 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 profile have 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 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 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 1050C for 5 minutes and then observed in the UV chamber for detection of spots at 560nm.

d. HPTLC instrumental conditions

HPTLC was performed on 10 cm × 10 cm Pre-coated Aluminium Sheets of Silica Gel 60 F₂₅₄ (Merck). Sample solution of about 10µl was 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 560 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 × 6mm.

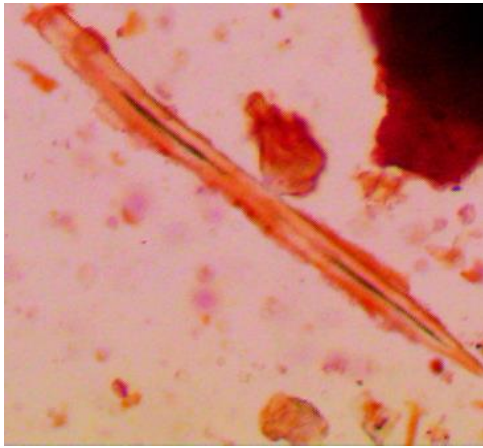
3.RESULT AND DISCUSSION**3.1 Macroscopic description:**

Jawarish-Ood-Sheerin is a dark brown semi-solid preparation having aromatic odour and sweet in taste.

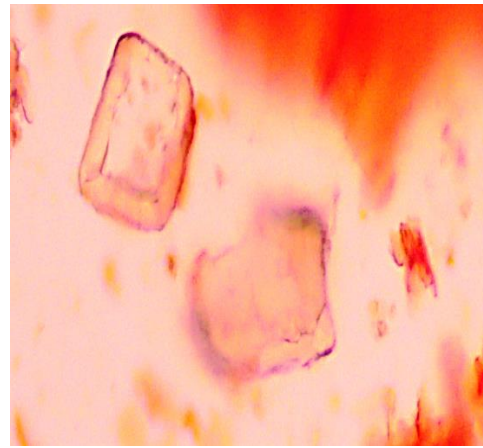
3.2 Pharmacognostical Observation (Powder microscopy)

Spindle shaped sclerenchyma fibre with very thick lumen, Stone cells with reddish content (**Darchini**); Starch grains with 4-5 components(**Jauzbuwa**); wood fibre having narrow lumen, medullary rays (**Ood hindi**); smooth spherical Pollen grain, spiral vessels (**Zafran**); fragment of perisperm, cells of seed coat (**Heel khurd**); Parenchyma cells with polygonal type of starch grains, Annular thickening of vessels, oleoresin cells (**Kulanjan**); stone cells, fragment of parenchyma,oil globules (**Filfil Daraz**);cells with suberized walls, vessels having pitted thickenings, starch grains with distinct centric hilum, annular vessels (**Asaroon**); Long fibres, prismatic crystals, group of stone cells (**Saleekha**).

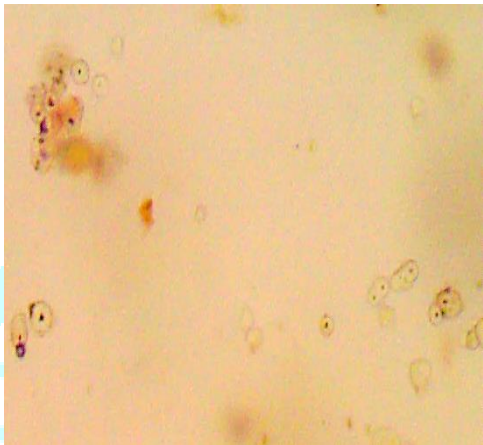
Figure 1(A-V)



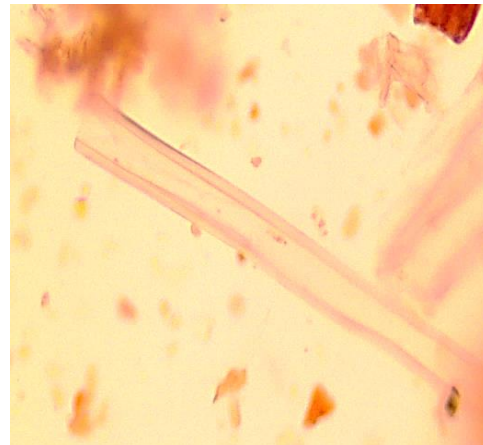
Darchini fibre A 10x



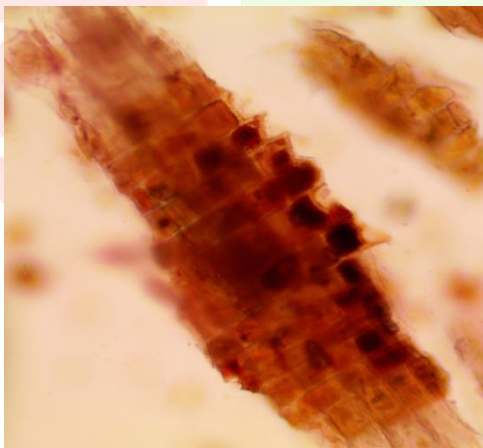
Darchini stone cells B 20x



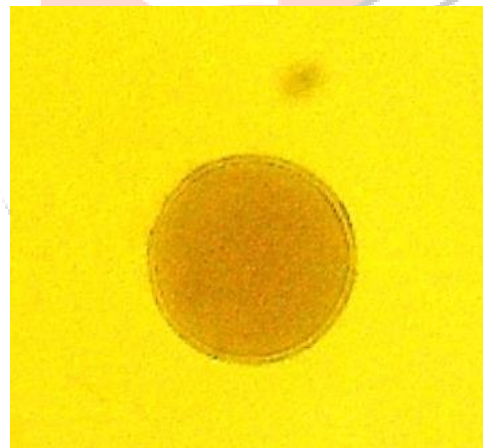
Jauzbuwa starch grains with 5-6 components 20x C



Ood hindi fibre D 20x



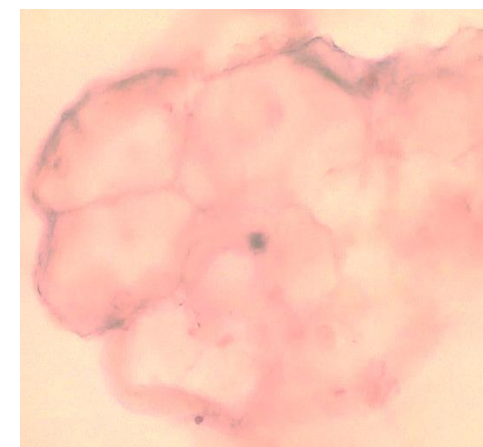
Ood hindi medullary rays E 20x



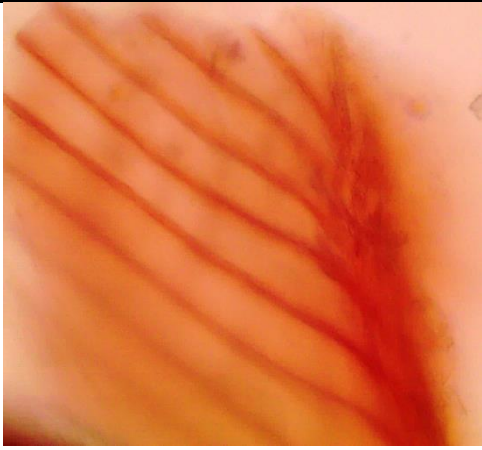
Zafran pollen grain F 20x



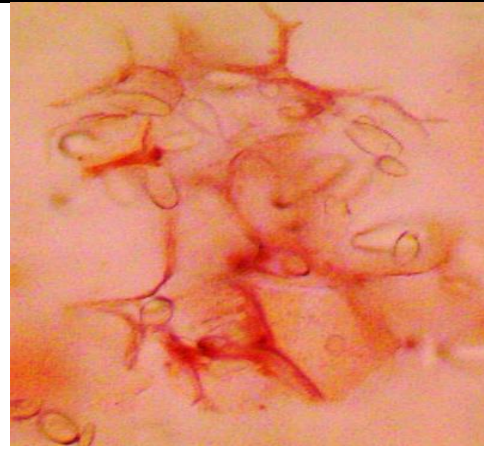
Zafran spiral vessels G 40x



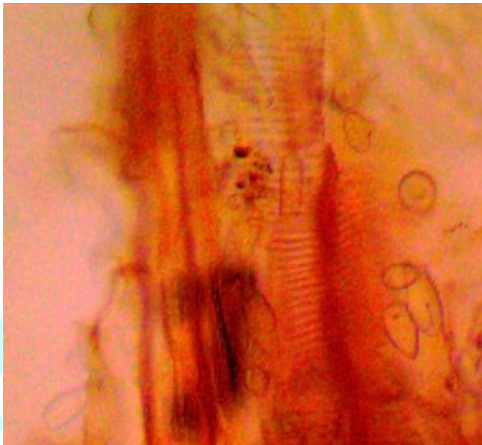
Heel khurd fragment of perisperm H 40x



Heel khurd cells of seed coat I 40x



Khulanjan parenchyma cell with starch grains 20x J



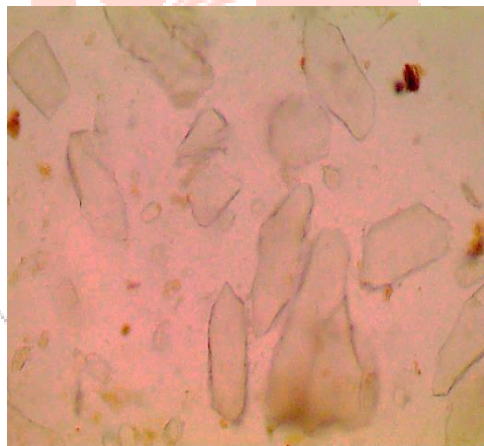
Kulanjan annular thickening of vessels 20x K



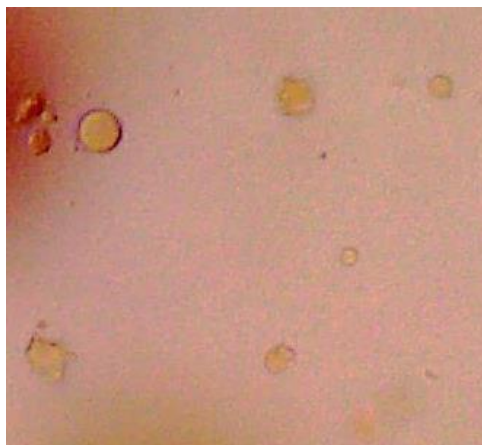
Kulanjan oleoresin cells L 20x



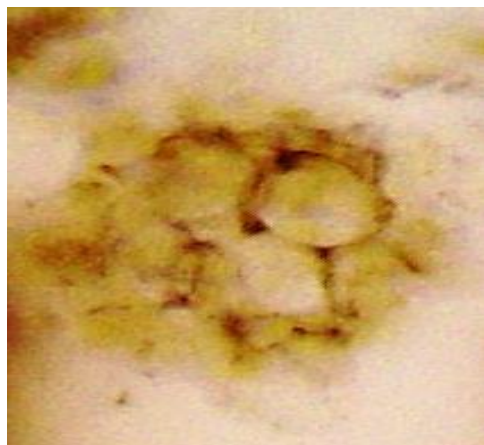
Filfil daraz stone cell M 20x



Filfil daraz fragment of parenchyma N 20x



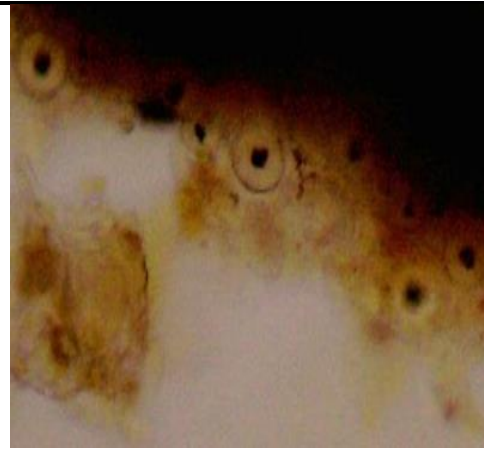
Filfil daraz oil globules O 40x



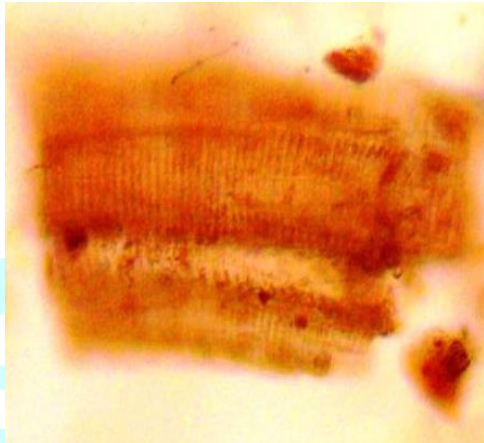
Asaroon cells with suberized walls P 40x



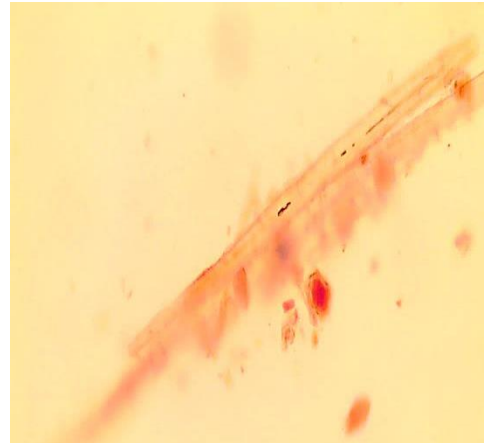
Asaroon pitted type of vessels Q 40x



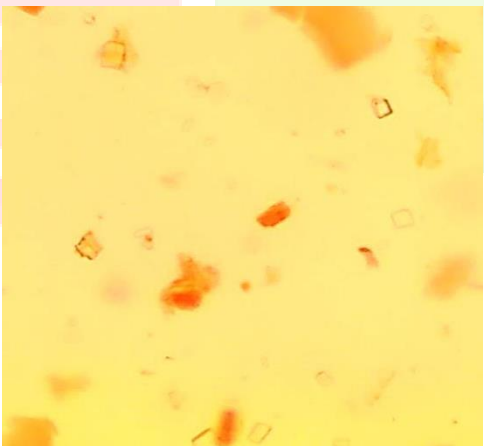
Asaroon starch grains with distinct centric hilum 40X R



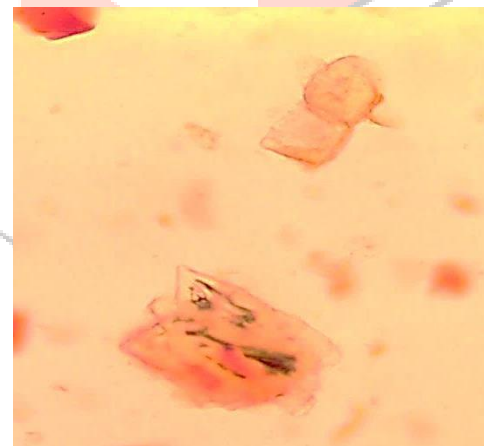
Asaroon annular vessels S 20x



Saleekha long fibres T 20x



Saleekha prismatic crystals U 20x



Saleekha stone cells V 20x

Figure 1.(A-V)

3.3 PHYSICO-CHEMICAL ANALYSIS

The Physico-chemical parameters of the formulations Jawarish-Ood-Sheerin were studied such as total ash, acid insoluble ash, solubility in water and alcohol, loss in weight on drying at 105 °C, and pH of 1% & 10% aqueous solution, the results are tabulated in table 2.

Table 2 : PHYSICO-CHEMICAL ANALYSIS

S.No	Parameter	Results
1.	Total ash (%w/w)	0.1%- 0.2%
2.	Acid insoluble ash (%w/w)	NIL
3.	Alcohol soluble matter (%w/w)	42.0%-43.5%
4.	Water soluble matter (%w/w)	76%-79%
5.	Loss in wt. on drying at 105 °C (%w/w)	4.5%-5%
6.	pH of 1% aqueous solution	4.5- 4.75
7.	pH of 10% aqueous solution	5.2-5.8

3.4 HPTLC Profile

a. High Performance Thin Layer Chromatography of Alcoholic extract:

TLC profile under UV 366nm showed two major peaks at Rf values 0.08, 0.54 and five minor peaks at various Rf values and under UV 254nm showed four major peaks at Rf values 0.29, 0.41, 0.51, & 0.99 and under visible region after derivatization with 1% Vannillin-Sulphuric acid showed four peaks at Rf values 0.08, 0.42, 0.53 and 0.99 on TLC plate. (Fig 2.A-C)

TLC of Alcoholic extract of Jawarish-Ood-Sheerin Track 1: Sample-I; Track 2: Sample-II; Track 3: Sample-III

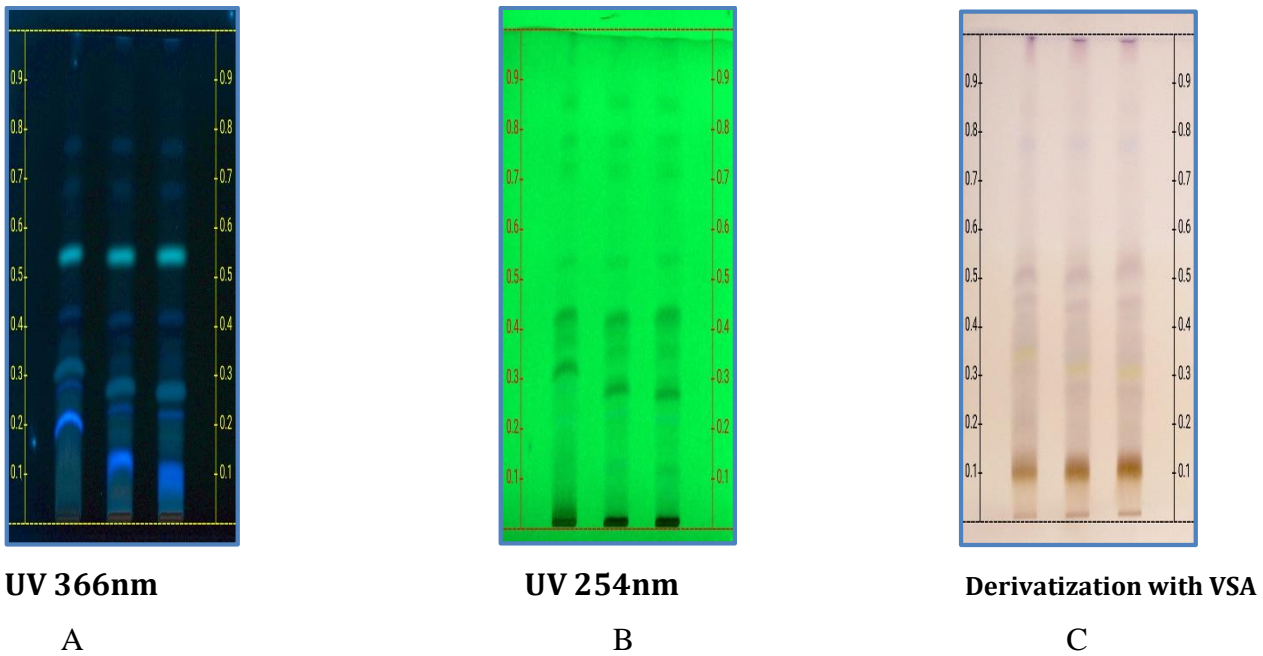


Figure 2(A-C)

HPTLC Fingerprinting Profile of alcoholic extract of Jawarish-e-Ood-Sheerin at UV366nm

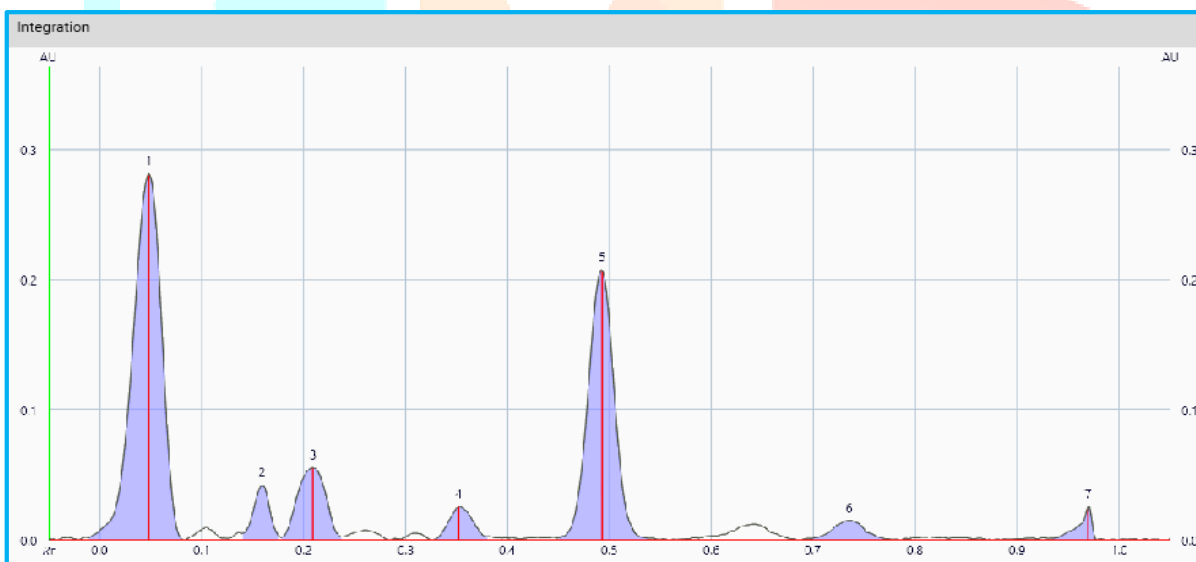
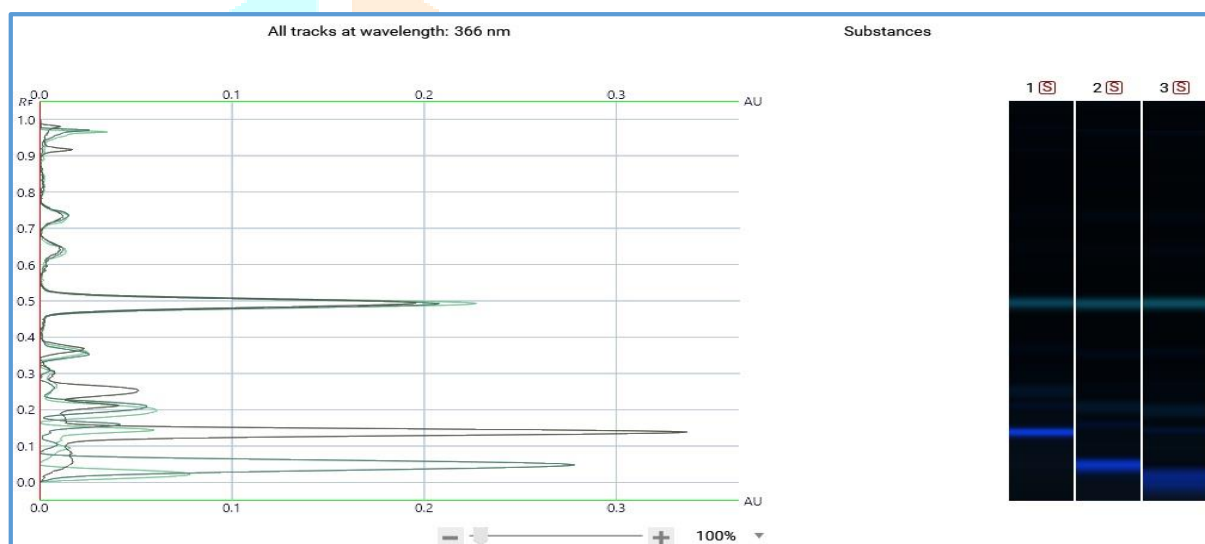


Figure 3

Table 3: Peak list of alcoholic extract of Jawarish-e-Ood-Sheerin at UV 366 nm.

Peak no	Area	Area %	Height	R _f value
1	0.00924	47.21	0.0001	0.08
2	0.00078	3.98	0.0021	0.18
3	0.00179	9.18	0.0023	0.24
4	0.00069	3.50	0.0026	0.38
5	0.00615	31.39	0.0012	0.54
6	0.00055	2.80	0.0002	0.78
7	0.00039	1.97	0.0000	0.98

HPTLC densitometry chromatogram of Alcohol extracts of Jawarish-Ood-Sheerin at 366 nm.**Figure 4**

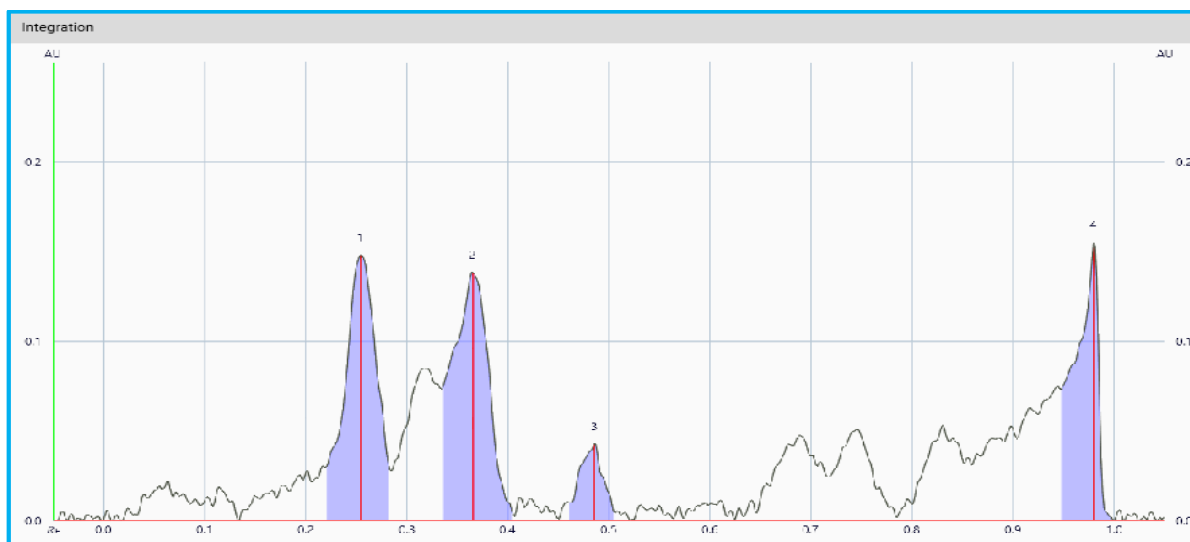


Figure 5

Table 4: Peak list of alcoholic extract of Jawarish-e-Ood Sheerin at UV 254nm

Peak no.	Area	Area %	Height	R _f value
1	0.00544	33.18	0.0278	0.29
2	0.00574	35.01	0.0031	0.41
3	0.00114	6.94	0.0040	0.51
4	0.00408	24.87	0.0016	0.99

HPTLC densitometry chromatogram of Alcohol extracts of Jawarish-Ood-Sheerin (03 batches) at 254 nm

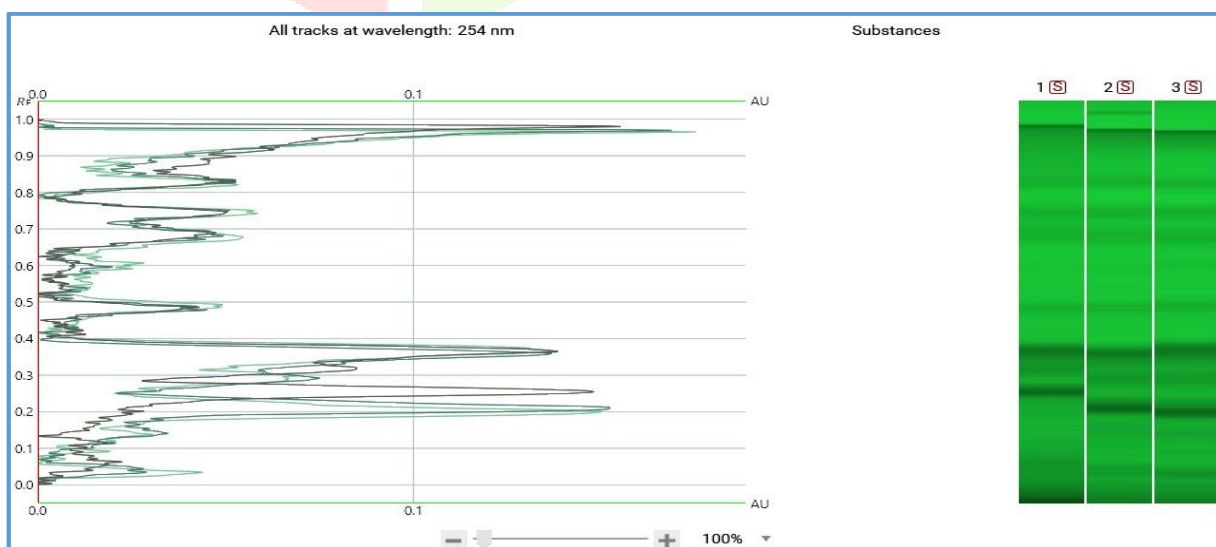


Figure 6

HPTLC Fingerprint Profile of alcoholic extract of Jawarish-Ood-Sheerin after derivatization with Vannillin- sulphuric acid at 560nm

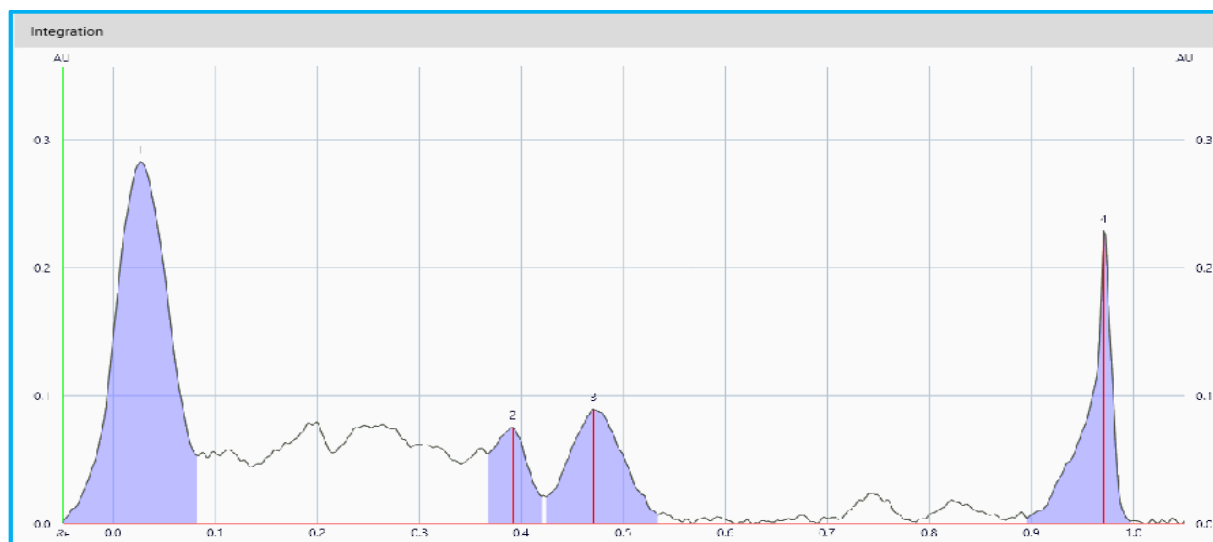


Figure 7

Table 5: Peak list of alcoholic extract of Jawarish-Ood-Sheerin after derivatization with Vannillin-sulphuric acid at 560nm

Peak no	Area	Area %	Height	R _f value
1	0.01773	54.82	0.0534	0.08
2	0.00295	9.12	0.0216	0.42
3	0.00558	17.24	0.0068	0.53
4	0.00609	18.83	0.0019	0.99

HPTLC densitometry chromatogram of Alcohol extracts of Jawarish-Ood-Sheerin after derivatization with Vannillin- sulphuric acid at 560nm

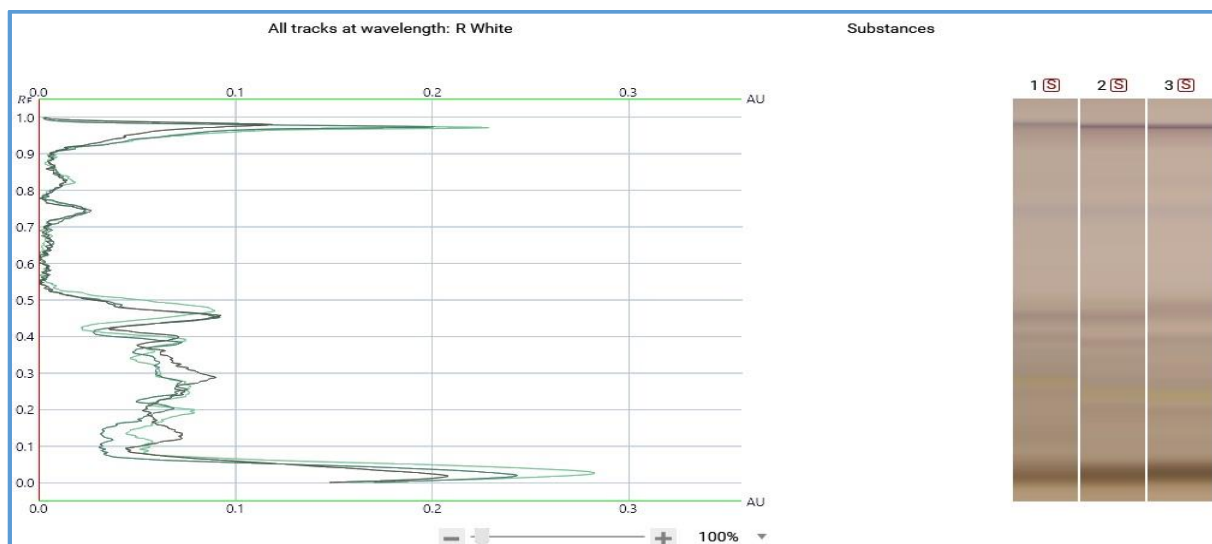
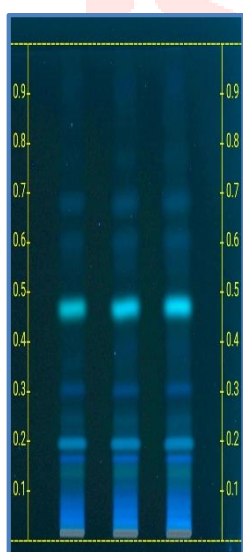


Figure 8

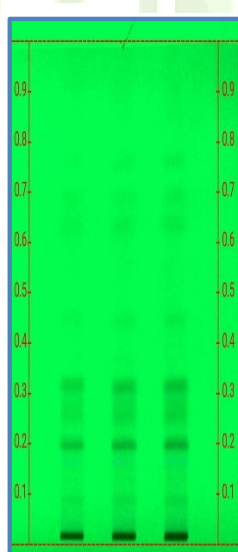
High Performance Thin Layer Chromatography of Chloroform extract:

TLC profile under UV 366nm showed six peaks at R_f values 0.00, 0.08, 0.13, 0.25, 0.45 & 0.68 and under UV 254nm showed four peaks at R_f values 0.01, 0.14, 0.27, & 0.96 and under visible region after derivatized with 1% Vannillin-Sulphuric acid showed seven peaks at R_f values 0.00, 0.05, 0.12, 0.17, 0.29, 0.37 & 0.64 on TLC plate. Figure 9 (A-C)

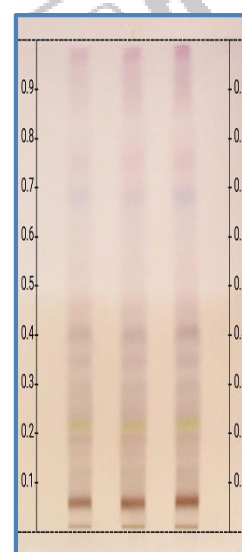
TLC of Chloroform extract of Jawarish-Ood-Sheerin Track 1: Sample-I; Track 2: Sample-II; Track 3: Sample-III



UV 366 nm
A



UV 254 nm
B



Derivatization with VSA
C

Figure 9 (A-C)

HPTLC Fingerprint Profile of Chloroform extract of Jawarish-Ood-Sheerin at 366nm

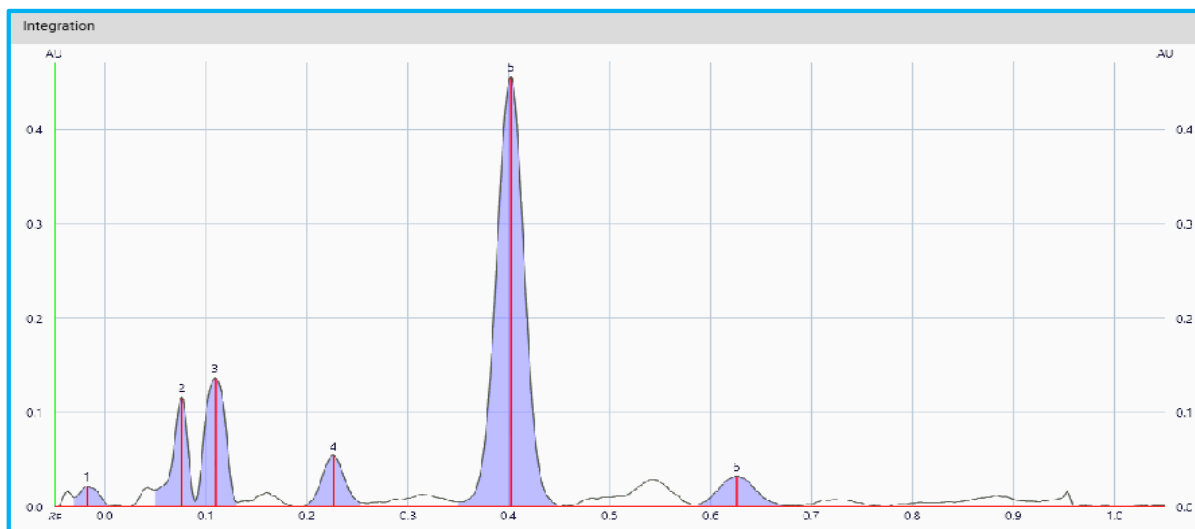


Figure 10

Table 6: Peak list of Chloroform extract of Jawarish-Ood- Sheerin at UV 366nm

Peak no	Area	Area %	Height	R _f value
1	0.00048	2.07	0.0017	0.00
2	0.00207	8.82	0.0049	0.08
3	0.00321	13.68	0.0044	0.13
4	0.00141	6.02	0.0039	0.25
5	0.01491	63.55	0.0021	0.45
6	0.00137	5.85	0.0012	0.68

HPTLC densitometry chromatogram of Chloroform extracts of Jawarish-Ood- Sheerin at 366 nm

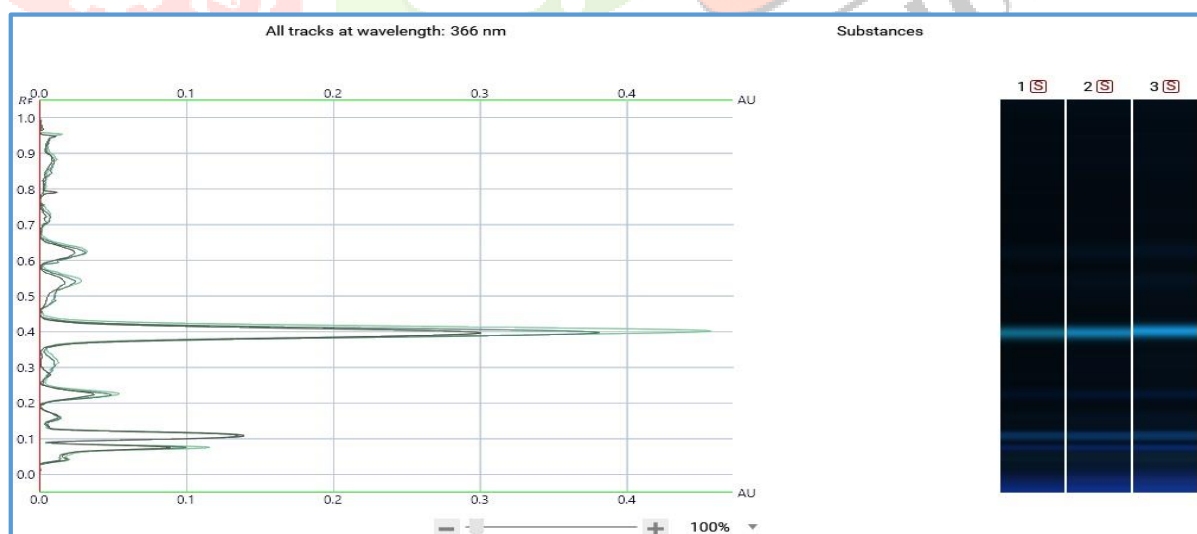


Figure 11

HPTLC Fingerprint Profile of Chloroform extract of Jawarish-Ood- Sheerin at 254nm

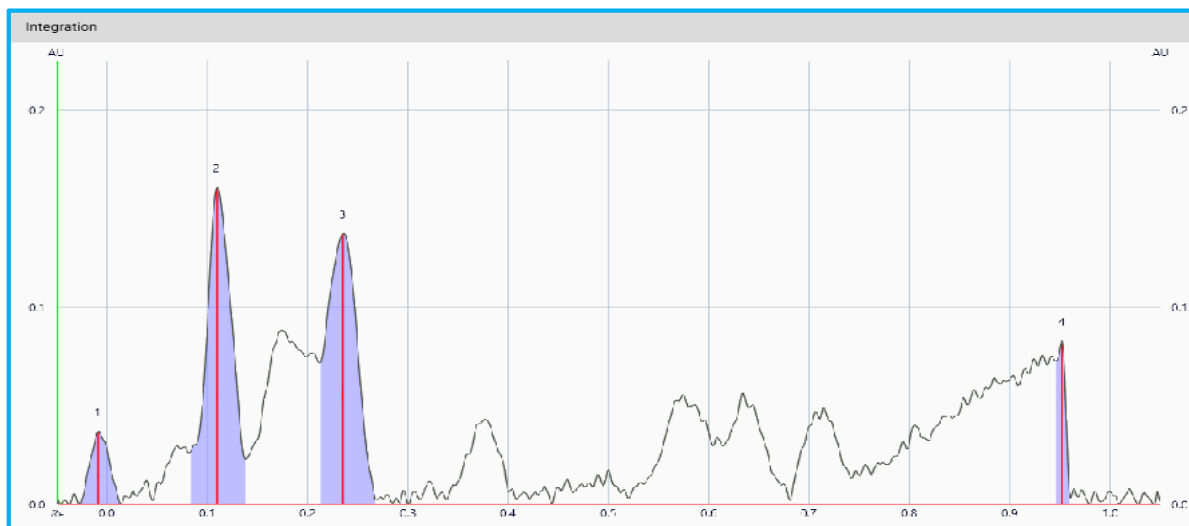


Figure 12

Table 7: Peak list of Chloroform extract of Jawarish-Ood- Sheerin at UV 254nm

Peak no.	Area	Area %	Height	R _f value
1	0.00080	7.16	0.0002	0.01
2	0.00473	42.39	0.0226	0.14
3	0.00481	43.11	0.0022	0.27
4	0.00082	7.34	0.0030	0.96

HPTLC densitometry chromatogram of Chloroform extracts of Jawarish-Ood -Sheerin at 254 nm

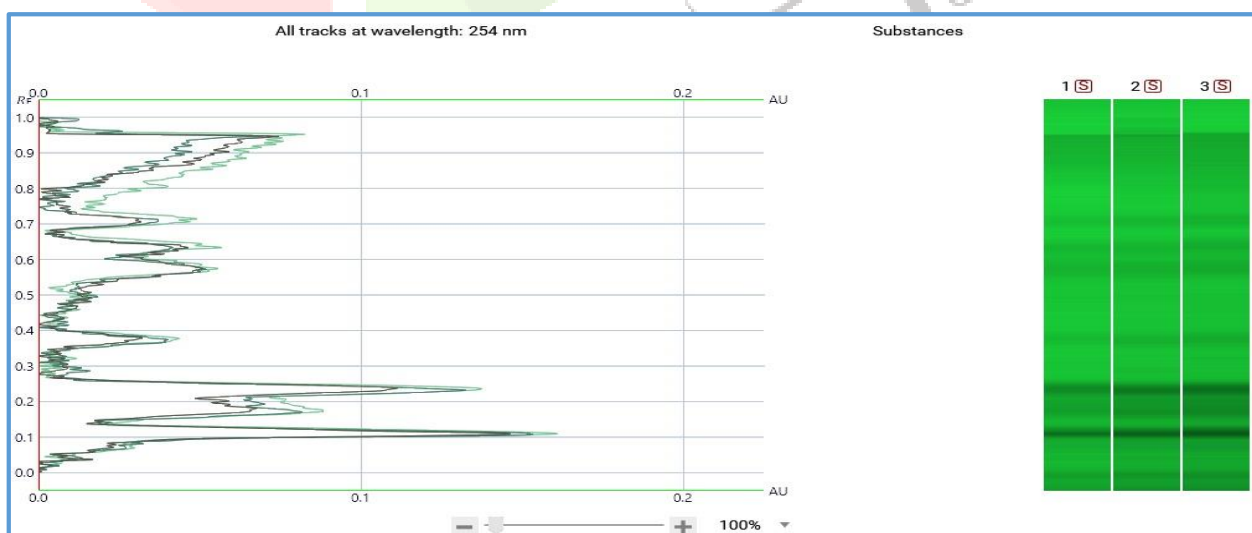


Figure 13

HPTLC Fingerprint Profile of Chloroform extract of Jawarish-Ood- Sheerin after derivatization with Vannillin- sulphuric acid at 560nm.

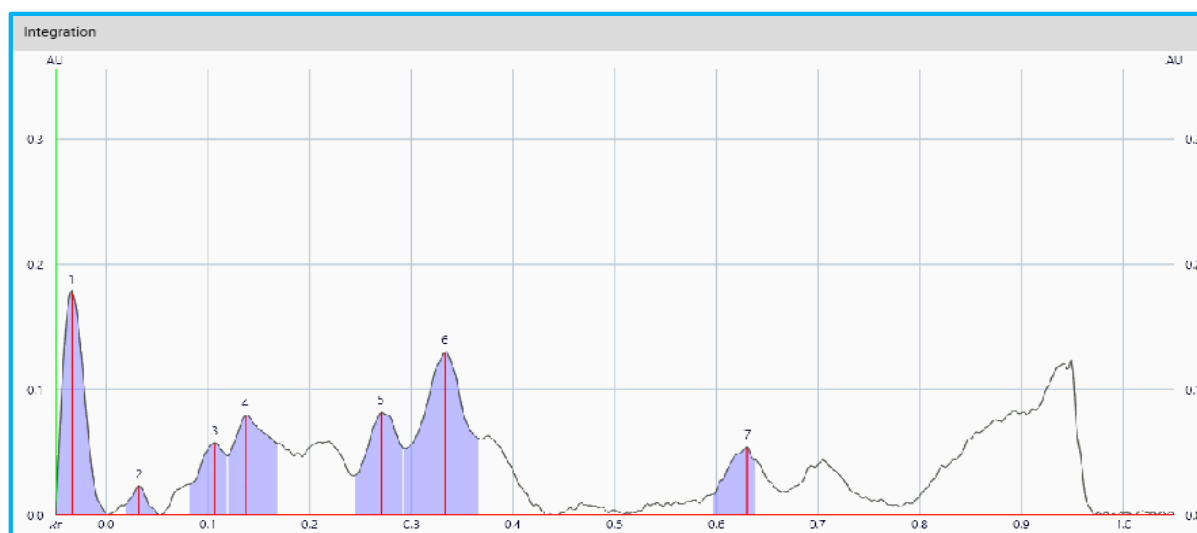


Figure 14

Table 8: Peak list of Chloroform extract of Jawarish-e-Ood Sheerin after derivatization with Vannillin- sulphuric acid at 560nm

Peak no	Area	Area %	Height	R _f value
1	0.00428	20.45	0.0000	0.00
2	0.00042	1.98	0.0000	0.05
3	0.00167	7.98	0.0470	0.12
4	0.00335	15.97	0.0564	0.17
5	0.00304	14.53	0.0527	0.29
6	0.00656	31.31	0.0604	0.37
7	0.00163	7.77	0.0436	0.64

HPTLC densitometry chromatogram of Alcohol extracts of Jawarish-Ood-Sheerin (03 batches) after derivatization with Vannillin- sulphuric acid at 560nm

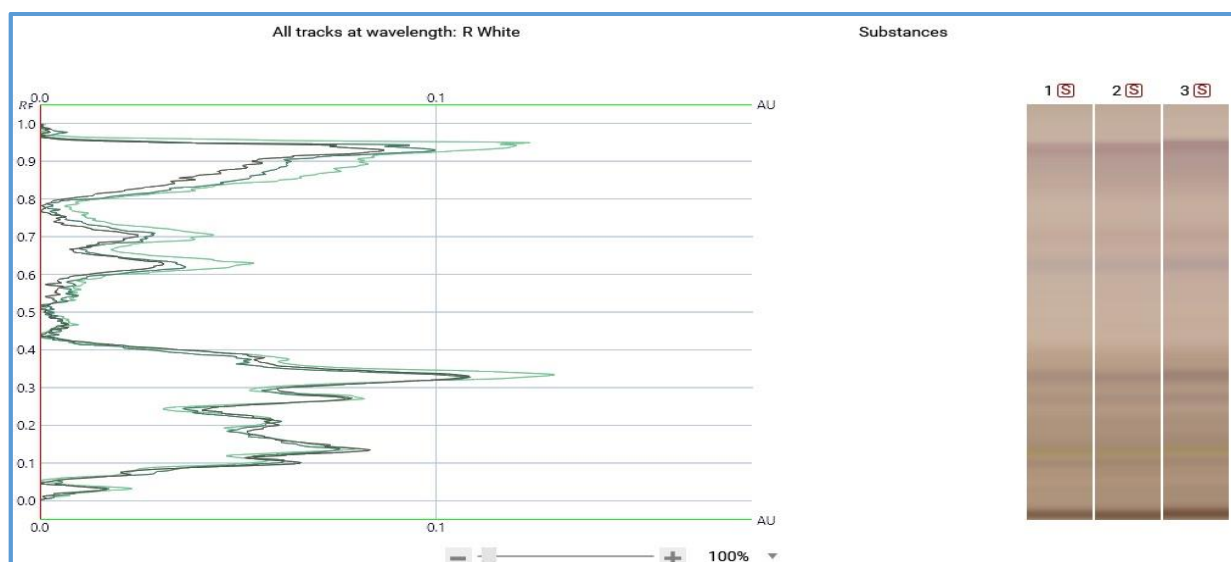


Figure 15

CONCLUSION

It can be concluded that organoleptic parameters are not much reliable in identification of polyherbal formulation as the ingredients are powdered and mixed together for preparing compound formulation. The present study therefore hold high significance as the microscopic features; various physico-chemical parameters, HPTLC profile etc. provide criteria for easy identification of the drug Jawarish-Ood-Sheerin and quality control analysis ensures the authenticity, quality and efficacy of the medicine.

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