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PHARMACOGNOSTIC EVALUATION AND HPTLC FINGERPRINTING OF ITRIFAL MULAYYAN-A UNANI POLYHERBAL FORMULATION

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ABSTRACT

Herbal medicines are gaining more and more attention all over the world due to their long historical clinical practice and less side effects. The major limitation with herbal medicines is that the lack of standardization technique which is crucial for evaluating the quality, purity, safety, and efficacy of drugs. Unani medicine is included within the Ayurveda, Yoga, Naturopathy, Unani, Homoeopathy (AYUSH) group of unconventional medical systems in India. People utilize Unani medicines as safe and effective treatments. *Itrifal Mulayyan* is a polyherbal unani formulation, which is commonly prescribed for treating various conditions such as chronic headache, constipation, cold, gastro-intestinal pain, catarrh, tinnitus, vertigo etc. The quality and authenticity of the unani formulation can be enhanced by using scientific methodologies. In the present study *Itrifal Mulayyan* formulation was evaluated using standard analytical procedures such as organoleptic characterization (colour, taste, aroma and consistency), macro & microscopic evaluation, physicochemical analysis, and high-performance thin-layer chromatography profiling and quality control parameters including microbiological load, heavy metals, aflatoxins, and pesticide residues. The parameters characterized in this paper may serve as reference standard for the quality control of *Itrifal Mulayyan* formulation.

KEYWORDS: Mulayyan, Physico-chemical, Pharmacopeial standard, WHO parameter, HPTLC, Unani medicine.

INTRODUCTION

Herbal medicines have existed world-wide with a long-recorded history, and they were used in ancient Indian**,** Chinese, Greek, and Egyptian medicine, for various therapeutic purposes.[1] The use of herbal products has increased globally due to their high demand in both developed and developing countries for primary health care purposes. Herbs and herbal formulations are generally considered to be attractive and economical alternative treatments because of their wide range of biological activities, perceived safety, ready availability, and lesser cost associated with the products.[2,3] The standardization and validation of herbal drugs is a challenging task as several factors influence the bio efficacy and reproducible therapeutic effects.[4] Contamination, adulteration, and the partial or total removal of costly ingredients or replacement of them are dangerous manufacturing procedures that eventually compromise the quality of the formulations.[5,6] Therefore, the safety and quality of herbal products should be ensured through pharmacovigilance, from proper identification of crude herbal drugs to collection season, extraction, purification process and rationalizing the combination in the case of poly-herbal drugs.[7]

AYUSH (Ayurveda, Yoga & Naturopathy, Unani, and Homeopathy) is India's unconventional medicine system based predominately on medicinal plants for therapeutic purposes. Unani medicine's origins can be traced back to the teachings of ancient Greek physicians Hippocrates and Galen.[8,9] The Unani medicines are being used by a large number of people worldwide as effective and safe remedies.[10,11] Although Unani system of medicine was originated in Greece, it is a well-recognized traditional system of medicine in India. In Unani medicine system, drugs are predominantly herbal, but also include animal, mineral, and metallic origins.[8,12,13] A number of polyherbal drugs in Unani medicinal system are being prescribed by Unani physicians for the treatment of a diverse range of chronic ailments for a long time.[8,9] *Itrifal Mulayyan* is one such unani formulation which have good laxative, analgesic, brain cleanser property.[14-16] Therapeutically, it is used for the treatment of *Suda'-e-Muzmin* (Chronic Headache), *Qabz* (Constipation), *Nazla* (Catarrh), *Zukam* (Cold), *Waja-e-Me'da wa Am'a* (Gastro-intestinal Pain), *Waj-ul-Uzn* (Otalgia), *Taneen* (Tinnitus), Duwar (Vertigo).[14,15,17-19] Quality control of medicinal plant products has been emphasized by the WHO by using modern techniques and appropriate practices. The present study aims to

establish pharmacopeial standards for *Itrifal Mulayyan* formulation by pharmacognostic investigations like macroscopic, microscopic examinations, physico-chemical analysis, HPTLC fingerprint and quality control such as heavy metal estimation, aflatoxins, microbial loads, and pesticide residue.

MATERIAL AND METHODS

Ingredients authentication

The formulation *Itrifal Mulayyan* was prepared at the Drug Standardisation Research Institute, Ghaziabad (DSRI). The drug was prepared using high-quality raw materials procured from the local market of Delhi and Ghaziabad, U.P. The botanist of DSRI authenticated the crude herbal drugs by using pharmacognostical methods.[20,21] Further, the ingredients were validated by comparing them to the monographs available in UPI (Part I), Vol. I, II, V & VII.[22] Table I shows the ingredients used to compose the formulation *Itrifal Mulayyan*.

Preparation of formulation

The *Itrifal Mulayyan* was prepared as per the standard method described in Unani Formulary of India (Table I).[15,23] All the ingredients were cleaned and dried under shade to remove the moisture if any. As per the literature, all the three Halelajat viz. Post-e-Bahera, Post-e-Halela Kabuli and Halela Siyah, pulverize separately and pass through 50 mesh sieves. Take all the three powders as per formulation of composition mix together and keep aside. Dissolve 75 g of Mastagi in 75 g of Raughan-e-Zard by subjecting to low heat (35-400) and stirring continuously. Add this dissolved Mastagi to Halelajat and mix thoroughly. Now, Prepare Qiwam by dissolving 2.25 Kg of sugar in 1.5 l of purified water and boiling till two tar consistency (68%) is obtained. Record the consistency using hand refractometer. Clean the remaining ingredients also, as above. Make their fine powder and pass through 50 mesh sieves, take them as per composition of formulation. Add all the ingredients to the Qiwam and mix thoroughly and heat the same to boiling for about 5-10 minutes, stirring continuously. Discontinue heating, and allow the same to cool to room temperature and fill it in the sealed container to protect from light and moisture.

Microscopy

About 5 grams of drug were gently stirred in hot water. After centrifuging the mixture, the supernatant was discarded. The sediment was washed and centrifuged several times and the supernatant was removed every time until a clear sediment was obtained. A small quantity of

the sediment was stained with iodine solution and mounted in 50% glycerin. Another small quantity was cleared with chloral hydrate solution, washed with distilled water and mounted in 50% glycerin. Different characters were observed in various locations.[24]

Physicochemical analysis

The physico-chemical parameters of *Itrifal-Mulayan* such as moisture content, extractive values in water and ethanol, ash values like total ash and acid insoluble ash, pH values in 1% &10% aqueous solution, bulk density and sugar content (reducing and nonreducing sugar) were examined using established techniques.[25,26]

High performance thin layer chromatography

Both polar and non-polar solvents, including ethanol and chloroform, were utilized to extract the formulation. After leaching out the sugar from the drug, the formulation sample of 2 gm each were sonicated with 30 ml of each ethanol and chloroform extract separately for about 20 minutes, and then filtered through Whatman No. 1 filter paper. The extracts were used for HPTLC fingerprinting after concentrating the extracts up to 10 ml under vacuum at 500C. Thin Layer Chromatography performed on an Aluminium TLC plate that has been pre-coated with silica gel 60 F_{254} (E. Marck) by employing CAMAG Linomat IV automatic sample applicator. With an automatic HPTLC sample applicator, apply 10µl of *Ethanol* extract on TLC plate as 10 mm band. With an automatic HPTLC sample applicator, apply 10µl of *Ethanol* extract on TLC plate as 10 mm band. In a twin trough glass chamber (10x10), the plate was developed up to a distance of 9 cm using 10 ml of the solvent system Toluene: ethyl acetate: formic acid (9: 1: 0.5) as mobile phase. The plate was viewed under UV light at wavelengths of 254 nm and 366 nm after being air-dried at room temperature. The plate was then heated to 105°C after being immersed in a 1% vanillin-sulphuric acid reagent until coloured bands started to form. Finally, the plate was examined under visible light. Similarly, 10µl of *Chloroform* extract was applied using a CAMAG Linomat IV automatic sample applicator to a separate aluminum TLC plate that had been previously coated with silica gel 60F254 (E. Merck). Rest of the process was repeated as carried out for ethanol extract.[27-29]

Quality control analysis

The quality control measure is necessary in order to ensure the safety and efficacy of finished herbal preparations. In present study various quality control measures, including microbiological load, heavy metals, aflatoxins, and pesticide residues, were analysed in order

to assess the quality of *Itrifal Mulayyan*. The examination of heavy metals and aflatoxins was done using, respectively, HPLC (Thermo Fisher) and Atomic Spectro photometer (LABINDIA).[30,31] GC- MS/MS system (ThermoFisher) by adopting QuEChERS method was used for the analysis of pesticide residue in accordance with standard procedure.[30-32] The microbial load was calculated using the recommended methodology.[32] In present study total aerobic bacterial count (TABC) and the total yeast and molds count (TYMC) were included for investigation. However, detection of Enterobacteriaceae members such as *Escherichia coli*, *Shigella* sp., *Salmonella* sp., *Klebsiella* sp. and specific objectionable pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* were all executed as per standard methods.[32]

RESULTS AND DISCUSSION

Organoleptic characteristics of formulation

The organoleptic characteristics are the fundamental criteria for choosing the right raw drug materials to ensure the quality of the final formulation. The characteristic observation of *Itrifal Mulayyan* were depicted at Table II.

Microscopic observation

The effectiveness of formulation is dependent on the authenticity of the herbs used. Authenticating herbs through anatomical studies is the first and fundamental step for standardization of herbal formulations. Microscopical identification of the herbal ingredients is a standard for statutory purposes in several solid and semisolid compound formulations. Formulation *Itrifal Mulayyan* consisted of various characteristic features of ingredients under microscope (Figure1a-u). These are fragments of epicarp surface, sclereids fibres, brachysclereid types stone cells of *Aamla*; epicarp in surface view showing stomata and reticulate parenchyma of the mesocarp of *Badiyan*; fragments of tracheid, macrosclereid stone cells, osteosclereid stone cells, and trichomes are characteristic feature of *Post-e-Bahera*. Pitted vessels and fragment of fibres of *Turbud Mausuf*; reticulate vessels and medullary rays of *Rewand Chini*; rosette of crystals, epicarp, sclerenchyma fibre of *Post-e-Halela kabuli /Halela siyah*; multicellular covering trichomes, wavy epidermal cells, pollen grains of *Ustukhuddusa*. Various mounts show some common characters of more than one crude drug like pitted vessels are common character of *Turbud Mausuf* and *Post – e- Balela*. Similarly, spiral vessels are common characters of herbal drug *Aamla, Post-e-Balela & Halela siyah*. Calcium oxalate crystals and starch grains are common characters of *Halela*

siyah, Post-e-Bahera, Turbud, Rewand Chini. All above mentioned characters were observed under various mounts are identifing microscopic features of the ingredients present in the formulation *Itrifal Mulayyan* which authenticate the presence of genuine crude drugs in the formulation.

Physicochemical analysis

The quantitative study of the data shows the formulation moisture content ranged between (13.16-14.78), which is ideal in the case of *Itrifals*. The low value of acid insoluble ash and a total ash level shows that the formulation was devoid of silicious matter. The water extractive values were found to be in range of 61.68-62.85% indicating a high sugar concentration. The ethanol extractive values were moderate ranged between (39.72-41.25%) which indicating the extraction of polar constituents. The aqueous extract of drug was slightly acidic in nature as pH values falls in the range of 5.31-5.54. Bulk density confirms the semi solid nature of the drug. Total sugar and non-reducing sugar are found to be expected range. Table III displays the physicochemical information of *Itrifal Mulayyan*.

HPTLC Profile

The use of HPTLC with various detectors is considered a powerful analytical tool especially for quality control of herbal medicines because of its simplicity and reliability. HPTLC fingerprints of both the extracts of *Itrifal Mulayyan* were observed under UV 254 nm, UV 366 nm and under visible light after derivatization. All the batches of *Itrifal Mulayyan* show similar colourful bands with similar Rf values. This shows the consistency of the formulation of the results (Figure 2 & 3).

Quality control parameters

Quality and safety issues may arise from the use of medicinal plants as raw materials for production due to proximity to wastewater application of fungicides and pesticides which the plant system may absorb or deposit superficially.[33-35] Presence of contaminants like heavy metals, pesticide residues, and microbiological safety evaluation are major issue in herbal medications and it is crucial to assess the toxicological risks of herbal drugs to ensure its safe human consumption.[36-38] The contamination of these herbal products leads to a reduction in their effectiveness and serious health hazards to consumers. So, the possibility of contamination of crude drugs used in formulation preparation cannot be ignored as it may have a severe impact on human life during the treatment process. Therefore, the heavy metal

content and microbial load of herbal products should be evaluated according to relevant scientific guidelines.

Microbial Load

A major issue in herbal medications is the microbiological safety evaluation. There are a number of factors that can cause microbial contamination in herbal medicines. The estimation of microbial growth is a crucial aspect of traditional remedies. The microbial load estimation can investigate whether or not the herbal drug contains disease-causing and spoilage microorganisms and if so, are they within the WHO's allowed limits? Present study was carried out to evaluate the total number of bacterial counts, total number of fungi, number of bacteria from the family Enterobacteriaceae, and number of pathogens such as *E. Coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Pseudomonas aeruginosa*. Bacteria belonging to the Enterobacteriaceae members viz. *Escherichia coli*, *Salmonella* sp., *Shigella* sp., Klebsiella sp. & specific objectionable pathogens such as Pseudomonas aeruginosa, *Staphylococcus aureus*, *Candida albicans* were not detected. Table IV show the microbial load data of the *Itrifal Mulayyan*, which are within acceptable levels, revealed that the medicine is safe for internal use or consumption.

Aflatoxin

Several molds, such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, produce aflatoxins, which can pose a serious threat to health. The results aflatoxins analysis showed that *Itrifal Mulayyan* did not show the presence of any aflatoxins (B1, B2, G1, or G2). Table V shows the result of aflatoxins analysis.

Heavy metal analysis

Heavy metals are harmful to human health and have been associated with various fatal disorders. The relative high density and atomic weight of a heavy metal make it dangerous or poisonous even when used in low concentrations. In present study heavy metal content in *Itrifal Mulayyan* was not detected which indicate its suitability for usage. The findings of the estimate of heavy metals are shown in Table VI.

Pesticide residues

Pesticides are chemical compounds used to control or eradicate pests. The presence of pesticide residues in herbal drugs is a consequence of agricultural practices, such as spraying and soil handling during farm operations.[39] So, it is quite challenging to harvest herbal

material as they may be easily contaminated by absorbing from soil, water and air. Pesticides can alter the quality, safety, and efficacy of herbal drugs. As per WHO rules, the herbal drugs should have safe levels of pesticide residues. In present study GC-MS was used to assess the pesticide residue. The study resulted that the drug is devoid of pesticide residue and is safe to use. The results of pesticide residues are shown in Table VII.

Table I: Formulation composition.

Sr. No.	Unani Name	Botanical name	Part Used
1.	Post-e-Halela Kabuli	*Terminalia chebula* Retz.	Ripe fruit Peel
2.	Post-e-Balela	*Terminalia belerica* Roxb.	Fruit Peel
3.	Halela Siyah	*Terminalia chebula* Retz.	Unripe dried fruit
4.	Aamla	*Emblica officinalis* Gaertn.	Fruit
5.	Turbud Mausuf	*Operculina turpethum* (L.) Silva Manso	Seed
6.	Badiyan	*Foeniculum vulgare* Mill.	Fruit
7.	Mastagi	*Pistacia lentiscus* L.	Gum
8.	Ustukhuddus	*Lavandula stoechas* L.	Inflorescence
9.	Saqmonia	*Convolvulus scammonia* L.	Latex
10.	Rewand Chini	*Rheum emodi* Wall. Ex. Meissn.	Root
11.	Asl	*Apis mellifera* L.	Honey

Table II: Organoleptic Characteristics of *Itrifal Mulayyan.*

S. No.	Parameters	Observation
1	Appearance	A semi-solid brownish-black preparation
2	Colour	Brownish-black
3	Touch	Smooth semi solid texture
4	Taste	Sweet
5	Smell	Pleasant

Table III: Physico-chemical parameters of *Itrifal Mulayyan.*

S. No.	Parameters	Results
1	Ethanol soluble matter (%)	39.72-41.25
2	Water soluble matter (%)	61.68-62.85
3	Loss in weight on drying at 105^0C (%)	13.16-14.78
4	Total ash (%)	1.25-1.56
5	Acid insoluble ash (%)	0.17-0.26
6	pH of 1% aqueous solution	5.31-5.54
7	pH of 10% aqueous solution	5.12-5.25
8	Bulk density	1.4658-1.4870
9	Reducing sugar	48.65-50.12
10	Non-reducing sugar	15.34-16.80

Table IV: Microbial load of *Itrifal Mulayyan*.

S. No.	Microbes analysed	Results
1	Total aerobic bacterial Count (TABC)	3.2×10^4 CFU/gm
2	Total yeast and molds count (TYMC)	5.1×10^2 CFU/gm
3	**Enterobacteriaceae members**	
4	*Escherichia coli*	ND
5	*Salmonella sp.*	ND
6	*Shigella sp.*	ND
7	*Klebsiella sp.*	ND
8	**Specific objectionable pathogens**	
9	*Pseudomonas aeruginosa*	ND
10	*Staphylococcus aureus*	ND
11	*Candida albicans*	ND
12	**Aflatoxin producing fungi**	
13	*Aspergillus flavus*	ND
14	*Aspergillus parasiticus*	ND

Table V: Aflatoxins level of *Itriphal Mulayan*.

S.N.	Parameter analysed	Result
1	$B_{1:}$	ND
2	$B_{2:}$	ND
3	G_1	ND
4	G2	ND

ND= Not detected

Table VI: Heavy metals analysis of *Itrifal Mulayyan*.

S. No	Metal analysed	Results
1	Arsenic	ND
2	Cadmium	ND
3	Lead	ND
4	Mercury	ND

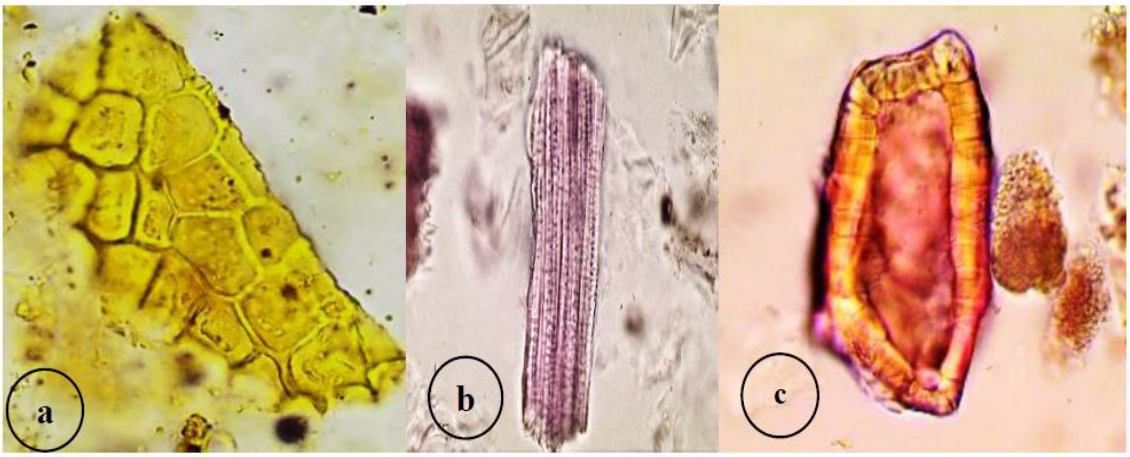
ND= Not detected

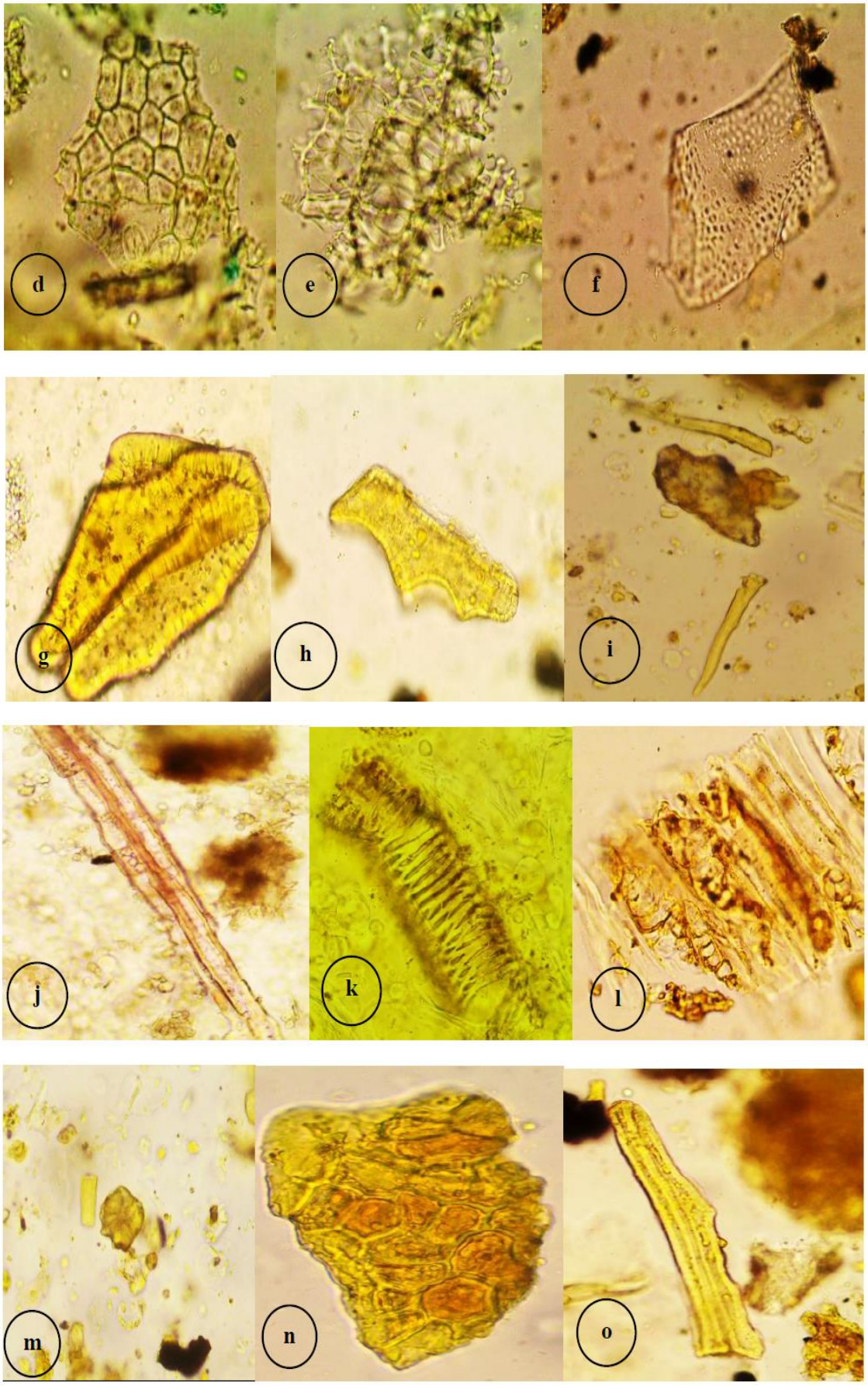
Table VII: Pesticide residues.

S. No.	Pesticide	Result (mg/Kg)	Permissible limit (mg/Kg)
1	Alachlor	BLQ	0.02
2	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	BLQ	0.05
3	Azinophos-methyl	BLQ	1.0
4	Bromopropylate	BLQ	3.0
5	Chlordane (cis, tans and oxychlordane)	BLQ	0.05
6	Chlorfenvinphos	BLQ	0.5
7	Chlorpyrifos	BLQ	0.2
8	Chlorpyrifos-methyl	BLQ	0.1
9	Cypermethrin (and isomers)	BLQ	1.0

10	DDT (all isomers, sum of p, p'-TDE (DDD) expressed as DDT)	BLQ	1.0
11	Deltamethrin	BLQ	0.5
12	Diazion	BLQ	0.5
13	Dichlorvos	BLQ	1.0
14	Dithiocarbamates (as CS2)	BLQ	2.0
15	Endosulphan (sum of isomers & Endosulphan sulphate)	BLQ	3.0
16	Endrin	BLQ	0.05
17	Ethion	BLQ	2.0
18	Fenitrothion	BLQ	0.5
19	Fenvalerate	BLQ	1.5
20	Fonofos	BLQ	0.05
21	Heptachlor (sum of Heptachlor & Heptachlor epoxide)	BLQ	0.05
22	Hexachlorobenzene	BLQ	0.1
23	Hexachlorocyclohexane isomer (other than γ)	BLQ	0.3
24	Lindane (γ – Hexachlorocyclohexane)	BLQ	0.6
25	Malathion	BLQ	1.0
26	Methidathion	BLQ	0.2
27	Parathion	BLQ	0.5
28	Parathion methyl	BLQ	02
29	Permethrin	BLQ	1.0
30	Phosalone	BLQ	0.1
31	Piperonyl butoxide	0.01	3.0
32	Pirimiphos methyl	BLQ	4.0
33	Pyrethrins (sum of isomers)	BLQ	3.0
34	Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	BLQ	1.0

***** BLQ – Below limit of quantification

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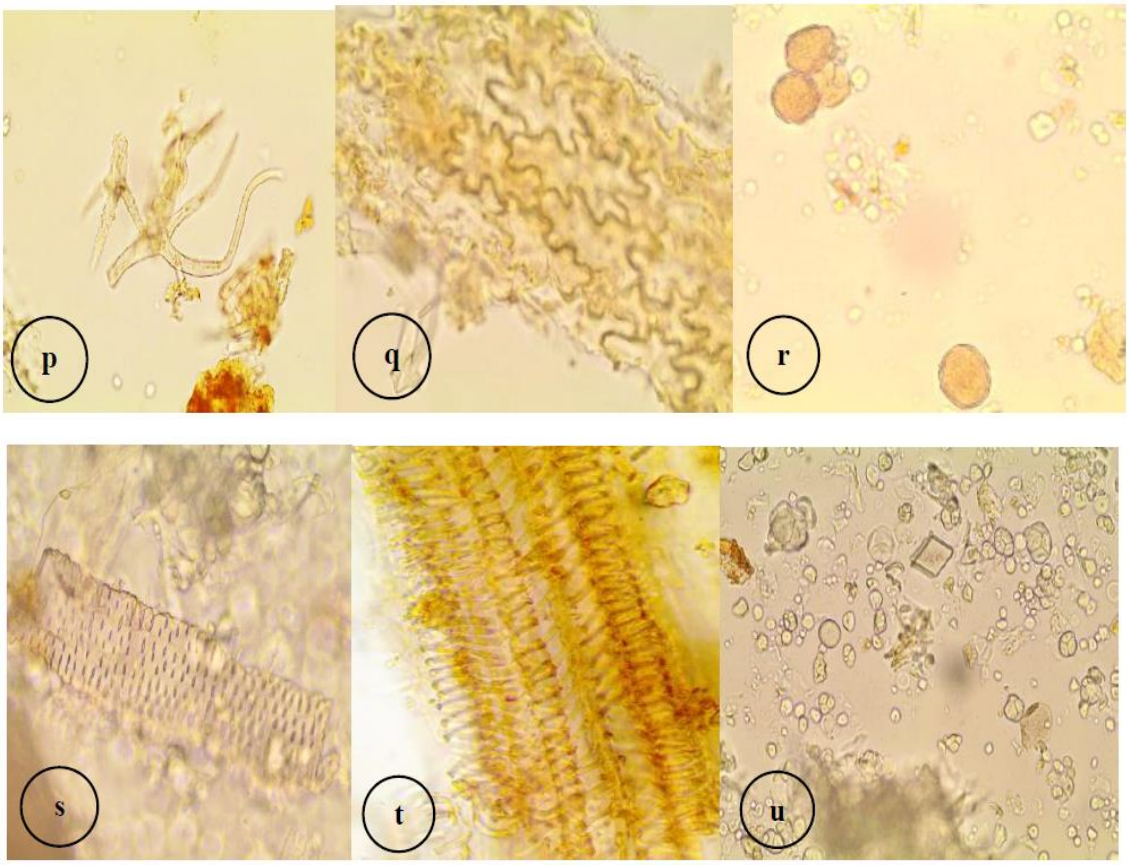


Figure 1a-u: Photographs of microscopic examination of compound formulation *Itriphal Mulayan*:- a,b,c) Epicarp surface, fragments of sclereids fibres, brachysclereids types stone cell (*Aamla*); d,e) epicarp in surface view showing stomata, reticulate parenchyma of the mesocarp (*Badiyan*); f,g,h,i) tracheid, macrosclereid stone cells, osteosclereid stone cells, trichomes (*Post-e-Bahela*); j) *fragment of fibres* (*Turbud Mausuf); k,l)* reticulate vessels, medulary rays (*Rewand Chini*); m,n,o) rosette of crystals, epicarp, sclerenchyma fibre *(Post-e-Halela Kabuli*/*Halela siyah*); p,q,r) multicellular covering trichomes, wavy epidermal cells, pollen grains (*Ustukhuddus);* s) pitted vessels (common character of *Turbud Mausuf and Post-e-Balela)*; t) spiral vessels (common characters of *Aamla, Post- e-Balela* & *Halela siyah*); u) starch grain and calcium oxalate crystals *(*common characters *Post-e-Halela Kabuli /Halela siyah, Post-e-Bahera, Turbud Mausuf, Rewand Chini*).

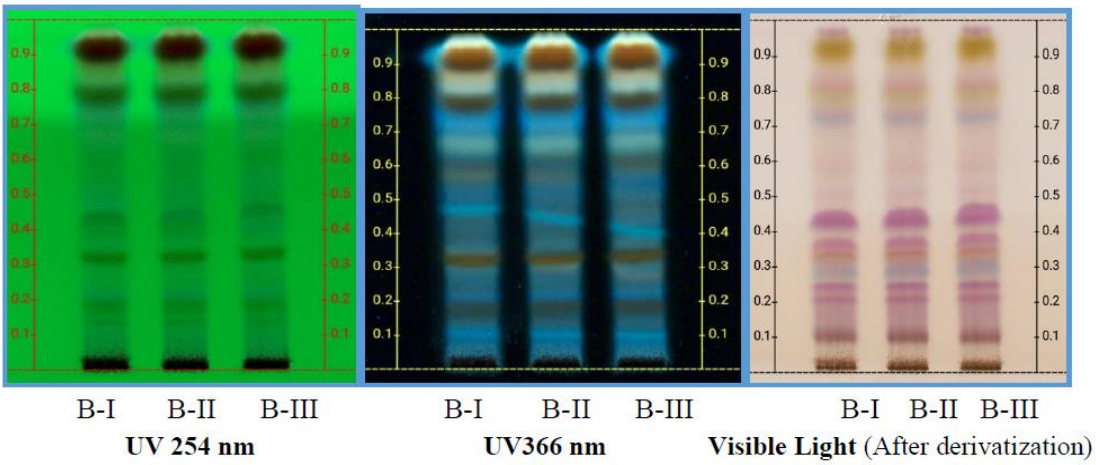


Figure 2: HPTLC of *Chloroform* extracts of *Itrifal Mulayyan*.

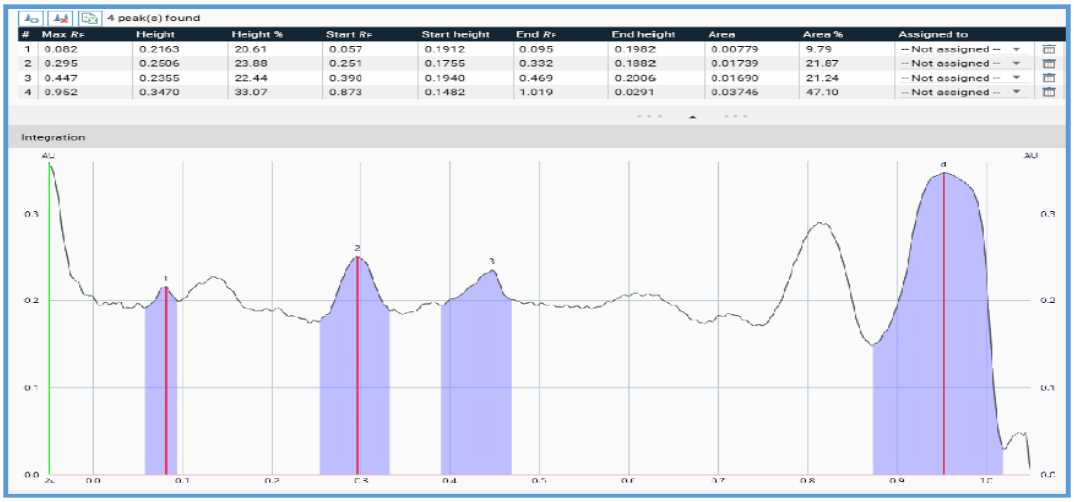


Figure 2a: HPTLC fingerprint profile of chloroform extract of *Itrifal Mulayyan* at 254 nm.

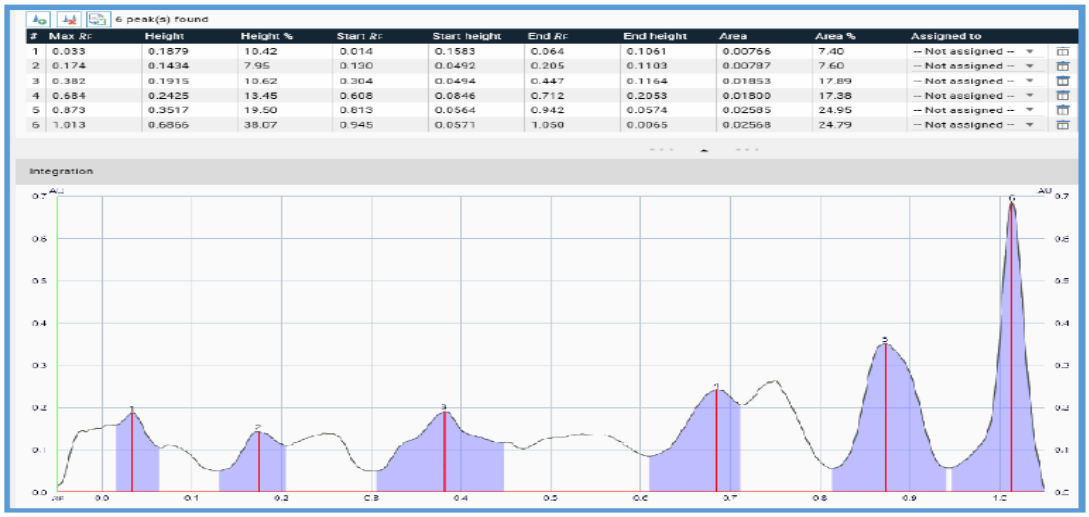
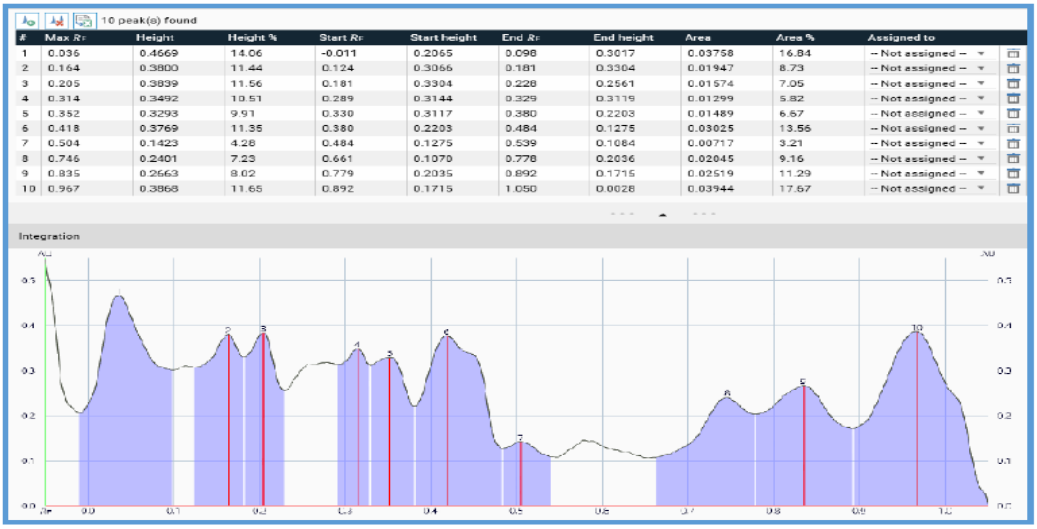


Figure 2b: HPTLC fingerprint profile of chloroform extract of *Itrifal Mulayyan* at 366 nm.



#	Max Rf	Height	Height %	Start Rf	Start height	End Rf	End height	Area	Area %	Assigned to
1	0.036	0.4669	14.06	-0.011	0.2065	0.098	0.3017	0.03758	16.84	-- Not assigned --
2	0.164	0.3800	11.44	0.124	0.3066	0.181	0.3304	0.01947	8.73	-- Not assigned --
3	0.205	0.3839	11.56	0.181	0.3304	0.228	0.2561	0.01574	7.05	-- Not assigned --
4	0.314	0.3492	10.51	0.289	0.3144	0.329	0.3119	0.01299	5.82	-- Not assigned --
5	0.352	0.3293	9.91	0.330	0.3117	0.380	0.2203	0.01489	6.67	-- Not assigned --
6	0.418	0.3769	11.35	0.380	0.2203	0.484	0.1275	0.03025	13.56	-- Not assigned --
7	0.504	0.1423	4.28	0.484	0.1275	0.539	0.1084	0.00717	3.21	-- Not assigned --
8	0.746	0.2401	7.23	0.661	0.1070	0.778	0.2036	0.02045	9.16	-- Not assigned --
9	0.835	0.2663	8.02	0.779	0.2035	0.892	0.1715	0.02519	11.29	-- Not assigned --
10	0.967	0.3868	11.65	0.892	0.1715	1.050	0.0028	0.03944	17.67	-- Not assigned --

Figure 2c: HPTLC fingerprint profile of chloroform extract of *Itrifal Mulayyan* under white light after derivatization.

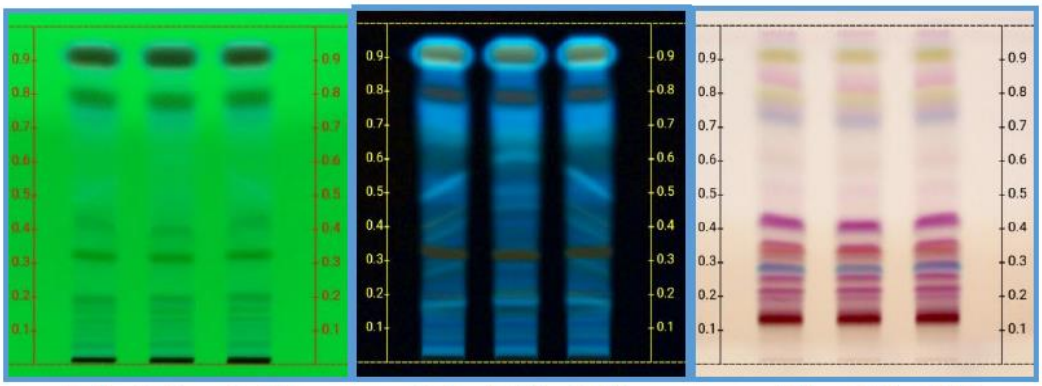


Figure 3: HPTLC of *Ethanol* extracts of *Itrifal Mulayyan.*

#	Max Rf	Height	Height %	Start Rf	Start height	End Rf	End height	Area	Area %	Assigned to
1	-0.024	0.1151	9.47	-0.043	0.0891	-0.002	0.0899	0.00423	6.69	-- Not assigned --
2	0.033	0.1101	9.05	0.022	0.0978	0.044	0.0995	0.00229	3.63	-- Not assigned --
3	0.057	0.1189	9.78	0.045	0.0989	0.070	0.0957	0.00270	4.26	-- Not assigned --
4	0.086	0.1207	9.92	0.071	0.0952	0.101	0.1051	0.00323	5.11	-- Not assigned --
5	0.126	0.1300	10.69	0.102	0.1049	0.156	0.0890	0.00608	9.62	-- Not assigned --
6	0.257	0.1497	12.31	0.216	0.0933	0.291	0.0979	0.00885	13.99	-- Not assigned --
7	0.364	0.1219	10.03	0.330	0.1005	0.436	0.0823	0.01089	17.22	-- Not assigned --
8	0.752	0.1502	12.35	0.696	0.0692	0.834	0.0283	0.01268	20.05	-- Not assigned --
9	0.885	0.1994	16.39	0.835	0.0281	0.932	0.0041	0.01229	19.44	-- Not assigned --

Figure 3a: HPTLC fingerprint profile of ethanol extract of *Itrifal Mulayyan* at 254 nm.

Figure 3b: HPTLC fingerprint profile of ethanol extract of *Itrifal Mulayyan* at 366 nm.

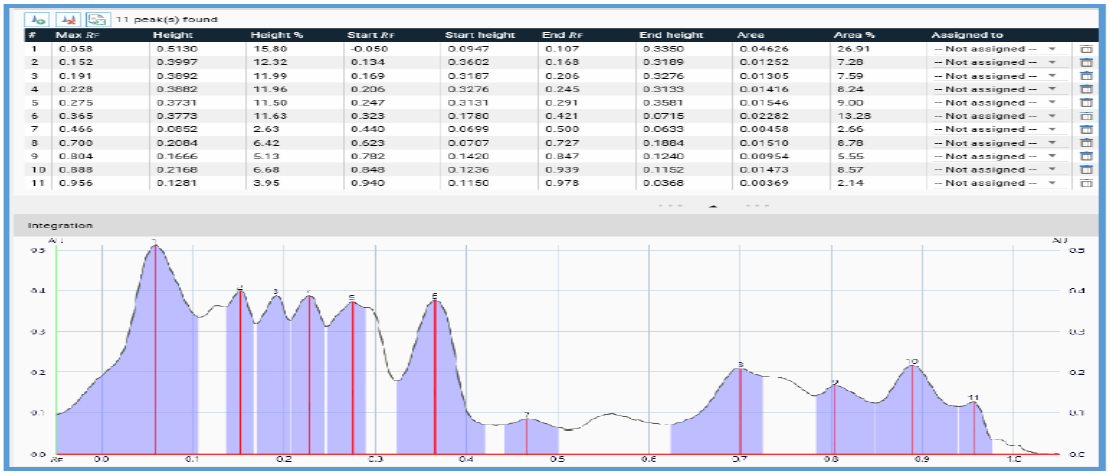


Figure 3c: HPTLC fingerprint profile of ethanol extract of *Itrifal Mulayyan* under white light after derivatization.

CONCLUSION

Ensure quality of herbal products by using modern techniques and implementing appropriate practices is a priority for the World Health Organization (WHO). Proper authentication and identification of single drugs as well as complex formulation is crucial with respect to their efficacy. In the present study pharmacopeial standards were employed to assess *Itrifal Mulayyan*, which undoubtedly assure the purity of the drug. All evaluated quality control parameters such pesticide residue, microbiological load, aflatoxins, and heavy metal were under the WHO permitted limit which signifying that the formulation is free of harmful ingredients and can be used safely. The study's findings can assist in standardizing the polyherbal Unani formulation *Itrifal Mulayyan*, which will increase its global adoption.

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