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CHROMATOGRAPHICAL AUTHENTICATION AND STANDARDIZATION OF UNANI HERBAL FORMULATION HABB-E-SURANJAN

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ABSTRACT

The Unani system of medicine has a long history of usage for the treatment of various diseases. During and after the COVID period, people realized its significance and a rapid growth has been observed in the usage of Unani medicine. This rise has posed novel challenges for proper authentication, safety and efficacy of Unani medicines. Hence, adoption of appropriate methodology is necessary to evaluate the quality of herbal medicines for their safe consumption. The Unani poly-herbal formulation; Habb-e-Suranjan, widely used as an effective analgesic for all types Waj-al-Mafasil (Arthralgia), Irq-un-Nisa (Sciatica), Niqras (Gout), Falij (Paraplagia), Laqua (Facial Paralysis or Ball's Palsay). The drug was studied through various standardization parameters such as organoleptic evaluation (color, odor and taste), physico-chemical evaluations (moisture content, total ash, acid insoluble ash, pH values, water and ethanol soluble extractive and volatile oil), with the chromatographic technique like HPTLC fingerprinting. The WHO quality control parameters such as heavy metals, aflatoxins, pesticide residues and microbial load were also carried out to check the presence of any hazardous substances in the formulation. The data produced in this study will lead to develop authentic pharmacopoeial standard of Habb-e-Suranjan which eventually leads to estimate the quality, safety and efficacy of the formulation.

KEYWORDS: Authentication, physico-chemical analysis, quality control parameters and HPTLC fingerprinting.

INTRODUCTION

Traditional medicine plays a very important role in human life for maintaining wholesome health.[1,2,3] The world health organization (WHO) also supports the traditional medicines usage and encourages the member countries to frame policies, regulatory and legal mechanism in order to implement for health care programme and ensuring authenticity, safety and efficacy of traditional medicine.[4] WHO has chosen India as integrated medicine center. The government of India has formed different organizations to regulate quality, safety and efficacy practices and documentation of herbal medicine (National policy on Indian system of medicine and homeopathy-2002).[5]

Standardization of herbal drugs is not an easy assignment as various components such as temperature, geographical locations, period and time of collection, age and part of the plant collected, method of collections and various other factors affect the efficacy and reproducible therapeutic effect.[6] Habb-e-Suranjan is herbal Unani formulation, categorized as Habb under Huboob section listed in the National Formulary of Unani Medicine.[7] Habb can be prepared by grinding the crude drugs into fine powder. Then this fine powder was mixed with a suitable solvent to make lubdi mass and small, round, and uniformly shaped pills are prepared from this lubdi mass weigh from one tenth of a gram to one gram. The finished product dried and stored in a dried container free from moisture. Habbs should neither be very hard nor very soft.[8] Habb-e-Suranjan, an important Unani formulation is reputed for its Anti-inflammatory (Mohallil-e-waram), Analgesic (Musakken-e-Alam) action and therapeutically used in Waj-al-Mafasil (Arthralgia), Irq-un-Nisa (Sciatica), Niqras (Gout), Falij (Paraplagia), Laqua (Facial Paralysis or Ball's Palsay) (9, 10, 11) The drug Habb-e-Suranjan also Known for its pharmacological actions such as Mushil-e-Bulgam (phlegmagogue), Mushil-e-Safra (Cholagogue), Mushil-e-Sauda (Melanogogue), Dafa-e-Nigris (anti-gout), Muqawwi-e-Asaab (Nervine tonic), Munawwin (hypnotic/soporofic) and Musaffi-e-Dam (Blood purifier).[12, 13]

Habb-e-Suranjan is composed of five herbal ingredients which individually have magnificent medicinal properties. All the ingredients of this formulation have analgesic, anti-inflammatory and pergative effect. The main ingredient Suranjan Shireen (*Colchicum luteum*) is used in arthralgia,[14] gout,[15] Sciatica,[16] intestinal ulcer, jaundice, Bone pain, sclerotic and all types of arthralgia[17] and hemorrhoids.[18]

The present research work aims to develop quality parameters and evaluate the data to lay down the pharmacopoeial standards such as organoleptic parameters and physico-chemical evaluations were carried out along with chromatographic studies like HPTLC fingerprinting. The WHO quality control parameters such as heavy metals estimation, aflatoxin, microbial load and pesticide residue were also analyzed in order to standardize the formulations.[19]

MATERIALS AND METHODS

Identification of ingredients

All the ingredients were procured from local raw drug dealer and were identified botanically using pharamcognostical methods.[20,21,22] All the ingredients were further validated by comparing with the monographs published in UPI Part 1 Vol. I, V, VI and VII.[23]

Preparation of formulation

The formulation was prepared at laboratory scale where ingredients (Table 1) were cleaned and dried under shade to remove moisture. All the ingredients were crushed separately in an Iron mortar pestle to obtain a coarse powder which were further processed in a grinder to obtain the fine forms. These fine powders were thoroughly mixed together and sieved through mesh no. 100 to prepare homogenous mixture. This homogenous mixture was mixed with a little amount of water to make lubdi mass. This lubdi mass was used to prepare Haboob by mechanical process. These haboobs were dried under shade and stored in a tightly closed glass container free from moisture.

Physico-chemical analysis

The physico-chemical parameters of Habb-e-Suranjan viz. moisture content, extractive values (solubility in water and ethanol), ash values (total ash and acid insoluble ash) and pH values (1% and 10% aqueous solution) and volatile oil estimation were analyzed as per standard methods.[24,25]

High performance thin layer chromatography

The drug samples (2g each) were extracted separately with 25 ml each of chloroform and ethanol by sonicating for 20 minutes and filtered. The extracts were concentrated; made up to 10ml in volumetric flasks and used for HPTLC fingerprinting. 6µl of each extract was applied on aluminum TLC plate pre-coated with silica gel 60 F254 (E. Merck) by employing CAMAG Linomat IV automatic sample applicator. The plate was developed up to a distance of 9cm in twin trough glass chamber (10x10), using 10ml of the solvent system Toluene:

Ethyl acetate: Formic acid (9:1:0.5) as mobile phase. The plate was air-dried at room temperature and observed under UV at the wavelength 254nm and 366nm. Further the plate was dipped in 1% vanillin-sulphuric acid reagent and heated at 105^{o}C till colored bands appeared. The plate was finally examined under visible light.[26,27,28]

Quality control analysis

The herbal medicines including Unani medicines are being used worldwide as effective remedies which have leads to concern over their quality assurance. So, the different quality control parameters like microbial load, heavy metals, aflatoxins and pesticide residues were carried out to verify the required quality of the formulation Habb-e-Suranjan. Estimation of microbial load was conducted as per standard method. Aflatoxins and heavy metal analysis were carried out by respective use of HPLC (Thermo Fisher) and Atomic Absorption Spectrophotometer (LABINDIA). Pesticide residues were analyzed using GC-MS system (Thermo) equipped with mass selective detector as per standard methods.[29,30,31]

RESULTS AND DISCUSSION

Observation: Habb-e-Suranjan is a dark brown color pill, hard in texture having characteristics odor and slightly bitter in taste. The drug did not show any filth, fungus and objectionable matter while the sample was spread on the petri dish.

Physico-chemical analysis

Physico-chemical data of the drug Habb-e-Suranjan are shown in table 2. The quantitative assessment of the data exhibit that the moisture content in the drug is in the range of 10.15 to 11.84 %. The water-soluble extractives range between 33.85 to 35.24 % shows the absence of any inorganic constituents. The ethanol soluble extractive is low and lies in the range of 4.82 to 5.75 %. The quantitative standards reveal the presence of negligible amount of silicious matter in the sample as the total ash (10.65 to 11.42 %) and acid insoluble ash (6.61 to 6.85 %) was found to be low. The aqueous extract of the drug was very slightly acidic as falls in the range of 5.12 to 5.82. the volatile oil is present in traces.

HPTLC Profile

HPTLC fingerprinting is sensitive, reliable and convenient tool for identification of crude drugs as well as complex compound formulations as the plant species produce distinct chromatograms. HPTLC images of both the extracts of Habb-e-Suranjan were observed under UV 254nm, UV 366nm and under visible light after derivatization. All the batches of

Habb-e-Suranjan show similar colorful bands with similar Rf values. Moreover, their densitograms are almost superimposed on each other. This shows batch-to-batch consistency of the formulation (Fig. 1A-F).

Quality control parameters

Microbial load

Estimation of microbial growth is very important parameter in traditional medicines. It indicates whether the drug contains disease causing and spoilage microorganisms in permissible limits. The assessment is done for evaluating the total bacterial count, total fungal count, count of bacteria belonging the Enterobacteriaceae family, count of pathogens like E. coli, to Staphylococcus aureus, Salmonella spp. and Pseudomonas aeruginosa. The results of microbial load are shown in table 3 which indicate that the drug is safe for internal use.

Aflatoxins

The results of aflatoxins analysis of the drug are given in Table 4. Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. The results do not show the presence of any of the aflatoxin contents (B1, B2, G1, and G2) in Habb-e-Suranjan (Table 4).

Pesticide residues

The results of pesticide residues are given in table 5. Harvest of herbal material without the use of pesticides is very difficult due to several factors. But as per WHO guidelines, the major concern is whether the drug contains pesticide residue in permissible limits or not. In order to estimate the pesticide residue, the drug was analyzed on GC-MS. The results indicated that the drug is free of pesticide residues and safe for use (Table 5).

Heavy metal analysis

The results of Heavy metal estimation are given in table 6. Heavy metals are hazardous to human health and may cause many fatal diseases. A heavy metal has relatively high density or atomic weight and is toxic or poisonous even at low concentrations (Table 6). The heavy metal content in Habb-e-Suranjan was found to be below detection limit which indicated that the drug was free from heavy metal contamination.

Table 1: Formulation composition.

S. No.	Ingredients	Botanical name/ English name	Part used
1	Suranjan Sheerin	*Colchicum luteum* Baker.	Rhizome
2	Post-e-Halela Zard	*Turminalia chebula* Retz	Semi-ripe Fruit Peel
3	Sham-e-Hanzal	*Citrullus colocynthis.* (L.)	Fruit Pulp
4	Muqil	*Commiphora mukul* Engl.	Rasin
5	Turbud Mausuf	*Operculina turpethum* (L.) Sliva Manso	Seed

Table 2: Physico-chemical parameters.

S. No.	Parameters	Results (Range)
1	Water soluble extractive (%)	33.85-35.24%
2	Alcohol soluble extractive (%)	4.82-5.75%
3	Loss in wt. on drying at 105 °C	10.15-11.84 %
4	Total ash (%)	10.65-11.42%
5	Acid insoluble ash (%)	6.16-6.85%
6	pH of 1% aqueous solution	5.64-5.82
7	pH of 10% aqueous solution	5.12-5.40
8	Volatile oil	Traces

Table 3: Microbial load.

S. No.	Microbial analysis	Results	WHO permissible limit
1	Total aerobic bacterial count (TABC)	4.1×10^4 CFU/gm	10^5 CFU/g
2	Total yeast and molds count (TYMC)	3.4×10^3 CFU/ gm	10^3 CFU/g
	Enterobacteriaceae members		
3	*Escherichia coli*	ND	ND
4	*Salmonella* sp.	ND	ND
5	*Shigella* sp.	ND	ND
6	*Klebsiella* sp.	ND	ND
	Specific objectionable pathogens		
7	*Pseudomonas aeruginosa*	ND	ND
8	*Staphylococcus aureus*	ND	ND
9	*Candida albicans*	ND	ND
	Aflatoxin producing fungi		
10	*Aspergillus flavus*	ND	ND
11	*Aspergillus parasiticus*	ND	ND

Table 4: Aflatoxins level.

S. No.	Parameter analysed	Result	WHO permissible limit
1	B1	Not detected	<2 pbb
2	B2	Not detected	<5 pbb
3	G1	Not detected	<5 pbb
4	G2	Not detected	<5 pbb

Table 5: Pesticide residue.

S. No.	Pesticides	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 and 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 and Heptachlor epoxide)	BLQ	0.05
22	Hexachlorobenzene	BLQ	0.1
23	Hexachlorocyclohexane isomer (other than ɤ)	BLQ	0.3
24	Lindane (ɤ- Hexachlorocyclohexane)	BLQ	0.6
25	Malathion	BLQ	1.0
26	Methidathion	BLQ	0.2
27	Parathion	BLQ	0.5
28	Parathion methyl	BLQ	0.2
29	Permethrin	BLQ	1.0
30	Phosalone	BLQ	0.1
31	Piperonyl butoxide	BLQ	3.0
32	Pirimphos methyl	BLQ	4.0
33	Pyrethrins (sum of isomers)	BLQ	3.0
34	Quintozene (sum of Quintozene, pentachloroanilene and methyl pentachlorophenyl sulphide)	BLQ	1.0

Table 6: Heavy metal estimation.

S. No.	Metal analysed	Result	WHO permissible limit
1	Lead	Not detected	10.00 ppm
2	Cadmium	Not detected	0.30 ppm

3	Arsenic	Not detected	3.00 ppm
4	Mercury	Not detected	0.10 ppm

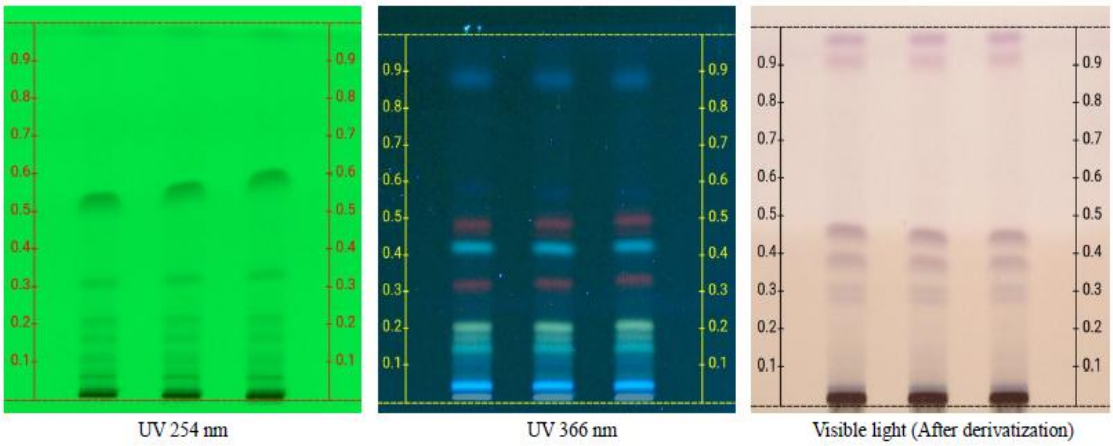


Figure 1A: HPTLC of ethanolic extract of Habb-e-Suranjan.

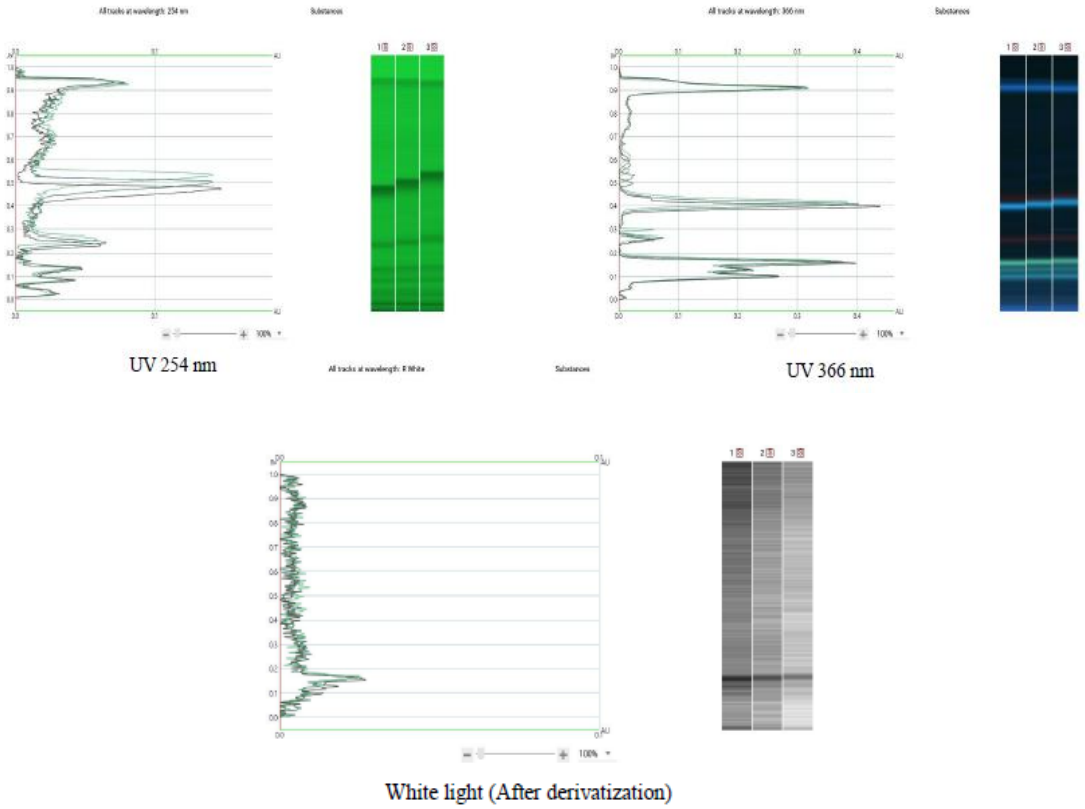
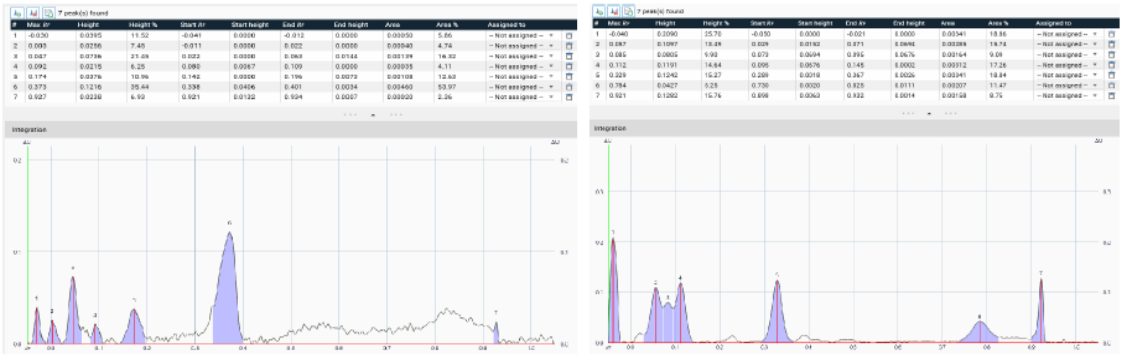
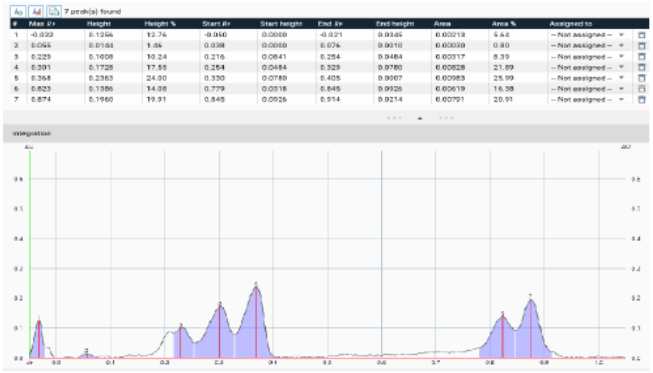


Figure 1B: HPTLC densitometry chromatogram of ethanolic extract of Habb-e-Suranjan.

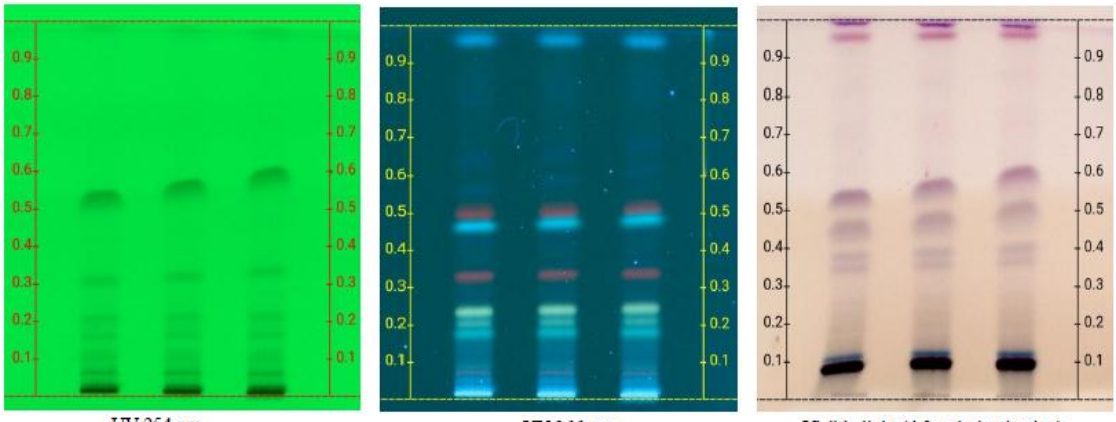


UV 254 nm UV 366 nm



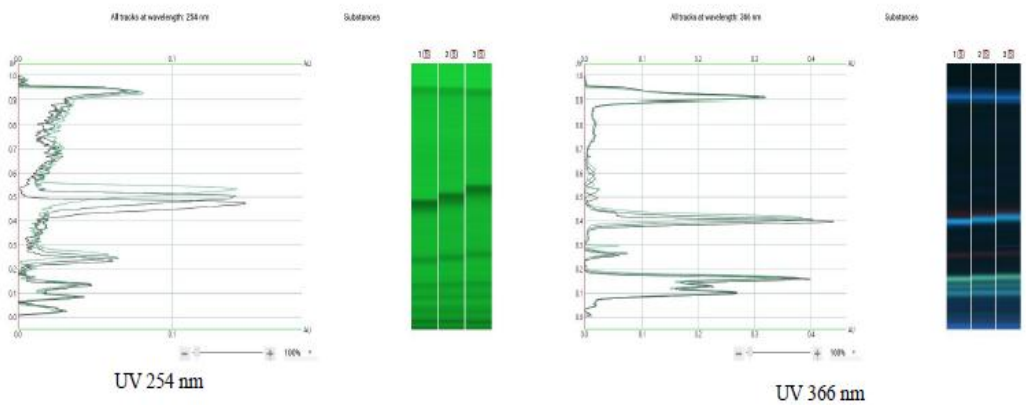
White light (After derivatization)

Figure 1C: HPTLC finger printing profile of ethanolic extract of Habb-e-Suranjan.



UV 254 nm UV 366 nm Visible light (After derivatization)

Figure 1D: HPTLC of chloroform extract of Habb-e-Suranjan.



UV 254 nm UV 366 nm

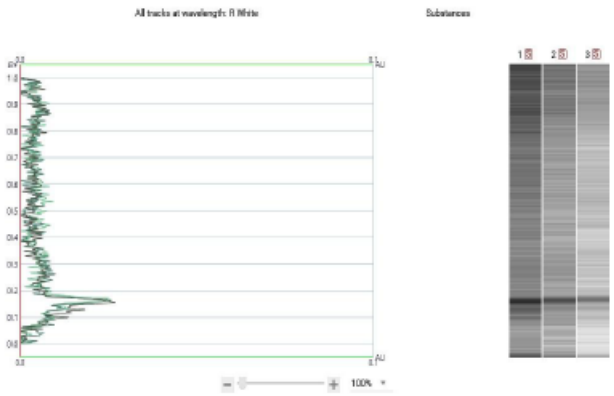
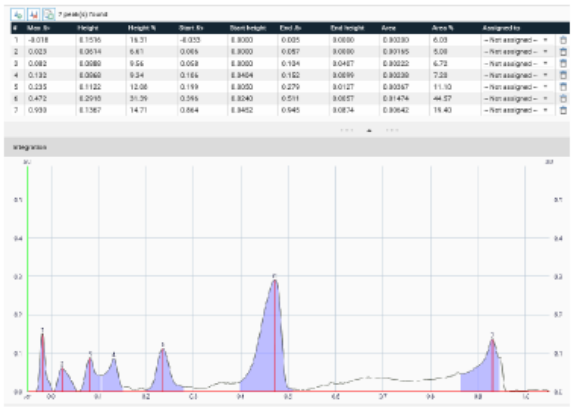
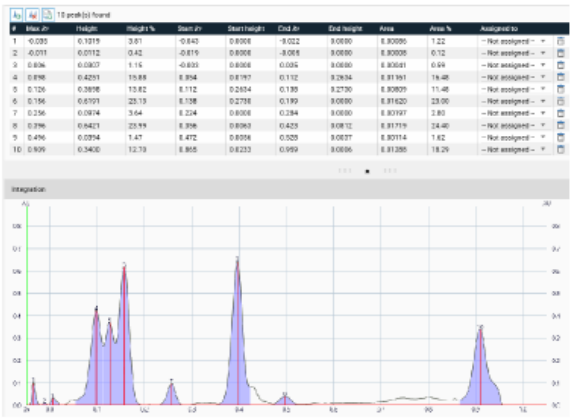


Figure 1E: HPTLC densitometry chromatogram of chloroform extract of Habb-e-Suranjan.





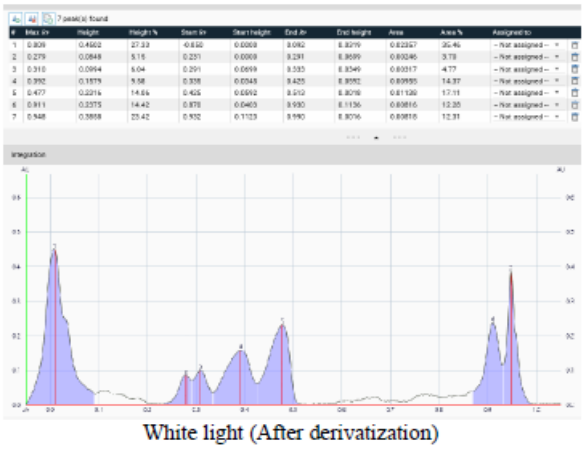


Figure 1F: HPTLC finger printing profile of chloroform extract of Habb-e-Suranjan.

CONCLUSION

Standardization of herbal medicine provides an assurance of its quality. Therefore, evaluation of physico-chemical, microbiological and quality control parameters aid to confirm the identity and purity of the herbal medicines. Habb-e-Suranjan was evaluated through pharmacopoeial parameters which certainly provides validation that the drug is safe for

internal use. HPTLC finger-printing also contribute for maintaining its authenticity. Hence, the present study ensures the quality and efficacy of the Unani formulation Habb-e-Suranjan.

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