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Quality control and HPTLC analysis of a polyherbal Unani formulation *Habb-i-Shahtara* recommended for skin diseases

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The Unani system of medicine (USM) is one of the popular traditional systems of medicine. World widely, this system of medicine becomes an important health care system. Since many years back Unani medicines were used by the Unani physicians for treating the diseases ailments. In USM most of the drugs are of plant origin and their therapeutic effects are due to the presence of active phytochemicals. The proper standardization methods must be necessary for preparation of formulations otherwise secondary metabolites or active constituents might not be up to the quality. The standard operating procedures (SOP's) are adopted for the preparation of Unani formulation. This study evaluated the quality of polyherbal unani formulation *Habb-i-Shahtara*, which is used in the treatment of skin diseases. The standardization parameters which were used for the development of quality standard of the study formulation were pharmacognostical studies, physicochemical and phytochemical parameters, HPTLC analysis, microbial load, aflatoxins, heavy metals and pesticide residues determination to evaluate the pharmacopoeial standard. The polyherbal Unani formulation *Habb-i-Shahtara* have been successfully standardized and the data and procedures which were used may serve the guidelines and standard reference in future for the preparation of formulation and for further study.

Keyword: *Habb-i-Shahtara*, HPTLC, Polyherbalunani formulation, Quality control, SOP's, Standardization, Unani

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In Unani system of medicine, medicinal plants play an important role in the treatment of different diseases for several decades. The use of herbal drugs continues nowadays due to their biomedical benefits. The medicinal efficacies of raw herbal drugs are based on the presence of one or more secondary metabolites which have physiological and pharmacological importance. Plants those have secondary metabolites *viz.*, glycosides, alkaloids, resins, terpenoids, tannins, steroids etc., are accepted because of their safety, efficacy, having lesser side effects and ethnic acceptability[1]. Presence of health and disease are based on the qualitative and quantitative balance or imbalance in four body *khilt* (*humours*) *viz., khilt-e-dum* (blood), *khilt-e-safra* (yellow bile), *khilt-e-sawda* (black bile), and *khilt-e-balgham* (phlegm), which plays an important role in the management of diseases and there are several Unani formulations which corrects the derangement of *humours* caused by ufūnat (infection) and these Unani drugs have been used since long by great Unani physician and scholar as these drugs have *Māni'i-'ufūnat* (anti-infective) properties and also helps in regaining the health. The study drug *i.e., Habb-i-Shahtara* was used by the Unani scholars as the oral dosage forms for the treatment of various skin diseases like *jarab-wa-hikka* (scabies and itching), *qūba* (tinea infection) and *ganj / sa'afa* (alopacia) which are caused by derangement in *khilt-i-safrā', khilt-i-sawdā'* and *khilt-i-balgham*[2-4]. The World Health Organization (WHO) admits the benefits and great importance of the herbal medicines used on a large scale of world's population for treatment of various ailments. As the uses of herbal medicine increasing day by day, so their safe and effective preparation and scientific evaluation has open the area for research and development into herbal medicine by using recent methods and applications. Unani system of medicine has the art of healing and outstanding past and wide reputation for the therapeutic efficacy and safety of its drugs[5]. In traditional medicine many plants and their products use in the form of concoctions, decoctions, infusions

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and plant extracts as medicinal substances, on the other hand many conventional drugs have been isolated from medicinal plants. In the present era plants are the major sources of medicinal use. Traditional knowledge of human health and medicines has recently become a major global concern. Traditional knowledge of medicinal plants is now considered to play a vital role in addressing the healthcare needs of developing countries and indigenous people. Traditional medicine is the best source of chemical diversity for finding new drugs and leads[6]. According to WHO about 88% of global population uses the traditional medicines for primary healthcare are derived from plants[7]. Nowadays, adulteration becomes common, and it is responsible for low quality of herbal drugs. The contamination with cheap, synthetic and earthy matter sometimes cross the permissible limit and completely replace the genuine drugs. Quality control (QC) of herbal drugs is of greater importance for preservation of quality of the natural herbs and products. Authentication and scientific validation of medicinal plant is a fundamental requirement of industry and other organizations dealing with herbal drugs. QC of botanicals, validated processes of manufacturing, customer awareness and post-marketing surveillance are the key points, which would ensure the quality, safety and efficacy of traditional medicine. For globalization of traditional medicine, there is a need for harmonization with respect to its chemical and metabolite profiling, standardization, scientific validation, documentation and regulatory aspects of traditional medicine[6,8].

The medicinal plants and their parts should be proved as genuine and free from deleterious substances like aflatoxins, pesticides, microbial contamination and heavy metals, etc., thus it is the urge of time to standardize the drugs on efficacy and safety standards to ensure that the drugs composed of medicinal plants should be in good quality. The integrity and accuracy of a drug is determined by the physicochemical analysis. The pharmacological activity of a drug is due to the presence or absence of the chemical constituents which is further determined by the qualitative analysis of the drug which estimate the presence of biological active substances like alkaloids, carbohydrates, glycoside, saponins, proteins, steroids, tannins, flavonoids etc. Now days it is the fact that the quality control of herbal drugs and their products are authenticated and identified by the chemical fingerprinting which are obtained by the chromatographic techniques[1].

Materials and Methods

Collection and authentication of plant material

The present research study was carried out at National Research Institute of Unani Medicine for Skin Disorders, Hyderabad (formerly known as Central Research Institute of Unani Medicine, Hyderabad). The raw ingredients used in the study drug were procured from a crude drug dealer in Hyderabad and their identification and authentication of were performed by pharmacognosist and botanist of NRIUM-SD, Hyderabad. The samples of ingredients were preserved in the Herbarium of the Department for future reference with voucher specimen number of Elwa / Sibr (SMPU/CRI-HYD14079), Halelasiyah (SMPU/CRI-HYD14080), Halelazard (SMPU/CRI-HYD14081), Shahtara (SMPU/CRI-HYD14082) and Saqmonia (SMPU/CRI-HYD14083).

Preparation of Unani formulation *Habb-i-Shahtara*

The study formulation *Habb-i-Shahtara* was prepared in GMP certified pharmacy of the department of IlmulAdvia (Pharmacology), NRIUM-SD, Hyderabad according to the composition of the formulation and method of preparation mentioned in Qarabadin-i-Qadri, GhinaMuna, National Formulary of Unani Medicine (NFUM) and Unani Pharmacopoeia of India (UPI)[9-12]. The study formulation *Habb-i-shahtara* consist of five ingredients *viz.,* Saqmonia (dried root extract of *Convolvulus scammonia L.*)[11], Aelwa (dried juice of leaves of *Aloe vera* L.), halelazard (dried mature Fruit of *Terminalia chebula* Retz.), halelasiyah (immature fruit of *Terminalia chebula* Retz.), Arq-i-shahtara (Leaves distillates of *Fumaria vailantii* Loisel) and all these crude drugs were taken in a proportion as mentioned in Unani literature[9,10]. The ingredients Saqmonia and Aelwa were detoxified separately as per the classical method mentioned in Unani literature[13-15]. Ingredients Halelazard and Halela Siyah were powdered in pulveriser to obtain a fine powder by passing through 80 number mesh[16]. The Saqmonia mushawwa (detoxified saqmonia) was powdered individually with the help of a metallic kharal to make fine powder. Halela Zard and Siyah were detoxified by charb method (roasting with oil) with roghan-i-badamshireen. The powdered ingredients mixed with aelwamushawwa

then arq-e-shahtara was poured in to the mixture, and mixed them properly then left to dry the mixture and again sufficient quantity of Arqe was mixed. When the composition becomes semisolid, a lubdy mass was prepared by which pills were manually prepared and this procedure was repeated in triplicate for three batches[9,10]. Prepared *Habb-i-Shahtara* were dried at room temperature and then kept in air tight container.

Organoleptic characters

Organoleptic characters of study formulation *Habb-i-Shahtara* such as appearance, shape, size, color, odour, taste and smell was carried out and depicted in Figure 1.

Powdered microscopy of the Unani formulation *Habb-i-Shahtara*

Microscopic examination of powder of *Habb-i-Shahatara* was carried out and microscopic characters were evaluated, for each batch of the Unani formulation. A few mg of the powder of study formulation was treated with 1% phloroglucinol along with concentrated hydrochloric acid (HCl) and mount in glycerin, iodine solution, chloral hydrate solution and Sudan III solution[17]. The samples of the three batches of study formulation were studied under electron microscope to rule out the peculiarities of the ingredient present in the study formulation *Habb-i-Shatara.*

Physico-chemical analysis

The physicochemical analysis of *Habb-i-Shahtara* were carried out under the analytical procedures which comprises of the observation of the organoleptic characters of the sample which includes appearance, color, shape, taste, odour, loss of weight on drying at 105°C, Total Ash Value, pH of 1% and 10% of aqueous suspension, Acid Insoluble Ash value, Alcohol Soluble Extract value, Water Soluble Extract value and disintegration time, friability value, hardness test, heavy metals detection, aflatoxins analysis, and microbial load assessment. The study formulation was also subjected to thin layer chromatography (TLC) techniques[18,19].

Fig. 1 — *Habb-i-Shahtara*

Qualitative phytochemical analysis

The phytochemical qualitative tests for the presence of Alkaloids, Glycosides, Carbohydrate, Phenols, Resins, Proteins, Steroids, Saponins, Tannins, Flavonoids, Starch and Fixed oils were carried out. The data obtained for the qualitative test were tabulated in.

Extracts preparation for HPTLC analysis

Accurately weighed 5 g powder of study drug was soaked in 200 mL of alcohol in a titration flask and was hold on for two hours at orbital shaker. The content was filtered using the No.41 Whatman filter paper and the filtrate was evaporated until it become concentrated up to 2 mL. The obtained solution was used as TLC sample. 10 µL alcoholic extract of drug was applied and various suitable solvent systems were used for the development of TLC and determination of various components of drug[18-20].

Development of HPTLC solvent system

10 µL of alcoholic extract of *Habb-i-Shahtara* was employed and different desirable solvent system was used for processing of sample. Solvent system for the TLC development was taken as Toluene: Ethyl Acetate: Methanol (7:2:1, v/v/v). The Spots were developed at 80 mm distance and the spots were then scanned under 254 nm and 366 nm wavelengths for the detection of different chemical constituents. The analysis was made on the bases of the number of spots and their Rf values[18,19].

Development of HPTLC technique

Pre-coated aluminum plates of silica gel 60 F_{254} (Merck, KgaA, Germany) was used for HPTLC analysis. The size of plate was of 20 cm X 10 cm and 200 X 100 mm with thickness of 0.2 mm. The 20 mm starting distance and 10 mm distance from bottom was maintained. 5 µL volume of sample extract was applied in the study and the HPLC grade solvent was used. During the study the extract was kept in a 5 mL

vials. 10 µL sample of the alcoholic extract of drug was administered in 10 mm width band by utilizing Automatic TLC Applicator System of the DESAGA SarstedtGruppe (Germany). Before HPTLC development a Twin Trough glass chamber was saturated with mobile phase vapors for 20 min at room temperature (25±2°C), and plate kept in glass chamber to continuous ascending development with Toluene: Ethyl acetate: Methanol (7:2:1, v/v/v). The development of solvent distance was found as 80 mm.

After developing the TLC plates were thoroughly dried up and explored by the appropriate detection system particularly in UV Cabinet system for detection of spots at 366 nm and 254 nm wavelengths under visible range which is depicted in the Figure 2. The plates were then scanned under the UV range of 366 nm and 254 nm wavelength separately and using visible region with the help of Densitometer CD60 of DESAGA SarstedtGruppe system. The densitogram obtained so faris demonstrated and display the peaks appearing for the corresponding spots being detected in the densitometer while scanning and the peaks areas under the curve correspond to the concentration of the component in the sample were revealed. The separation of the components of study formulation was repeated in triplicates for three batches and scanned.

Determination of heavy metals

Habb-i-Shahtara was taken for the detection of of heavy metals by Atomic Absorption Spectrophotometer method in Drug Standardization

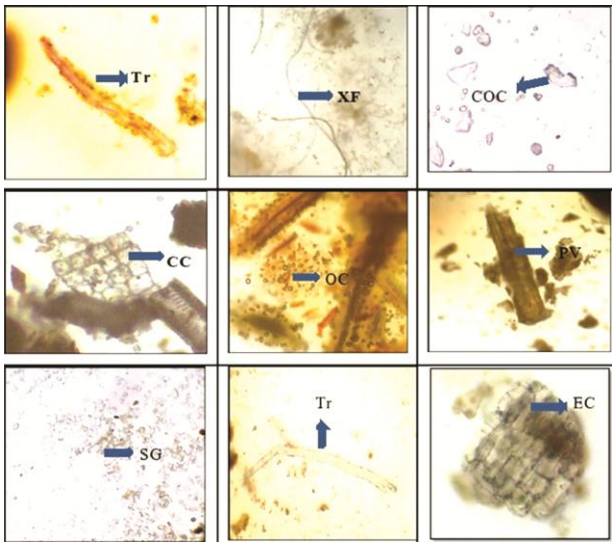

Fig. 2 — Powder microscopic observation of *Habb-i-Shahtara* Tr. Trichomes, XF. Xylem fibres, COC. Calcium Oxalate Crystals, CC. Cork cells, OC. Oil cells, PV. Pitted Vessels, SG. Starch Grains, Tr. Tracheids, EC. Epidermal cells

Research Institute under CCRUM at Ghaziabad. Detection of Lead (Pb) & Cadmium (Cd) was done by Flame atomization technique and Hydride generator was used for the detection of the elements like Arsenic (As) & Mercury (Hg).

Microbial load determination

The assessment of microbial load for *Habb-i-Shahtara* was performed as per the WHO /UPI guidelines[16,21].

Microbial count

The microbial counts were performed by the petri plate inoculated with the study formulation and it was taken at 24-48 h for Soyabean Casein Digest Agar Media, HiCrome *E. coli* Agar Media and Modified Salmonella Agar media, whereas plates of Sabouraud Dextrose Agar Media were read after 48-72 h.

Aflatoxin determination

The study formulation was determined for the presence of Aflatoxins by using the thin layer chromatography method. The accurately weighed quantities of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg /mL each for detection of aflatoxin B_1 and G_1 and 0.1 µg per mL each for detection of aflatoxins for B_2 and G^2 respectively[12,16].

Pesticides residue determination

The study formulation *Habb-i-Shahtara* was analysed for pesticides residue at Bureau Varitas India Testing Services Private Limited, Industrial Estate, Sanathnagar Hyderabad, 500018, Telangana.

Results

Organoleptic characters

Organoleptic characters of study formulation *Habb-i-Shahtara* depicted in Table 1.

Table 1 — Organoleptic characters of Unani formulation *Habb-i-Shahtara*

S. No.	Organoleptic Properties	Batch I	Batch II	Batch III
1	Appearance	Pills	Pills	Pills
2	Shape	Circular	Circular	Circular
3	Size	Diameter 5-7 mm	Diameter 5-7 mm	Diameter 5-7 mm
4	Color	Dark Brown	Dark Brown	Dark Brown
5	Odour	Unpleasant	Unpleasant	Unpleasant
6	Taste	Pungent followed by Bitter	Pungent followed by Bitter	Pungent followed by Bitter
7	Texture	Hard and Smooth	Hard and Smooth	Hard and Smooth

Microscopic observation

Powder of study drug shown as dark brown of colour, smooth texture, pungent odour, astringent and bitter taste. Xylem fibres, tracheids, pitted vessels are sometimes associated with a layer of oil cells, unicellular and uniseriatetrichomes, small microsette crystals of calcium oxalate and oil cells were revealed. Cells containing simple starch grains were found and shown in Figure 2.

Physico-chemical analysis

The physicochemical evaluations of *Habb-i-Shahtara* were carried out under the UPI prescribed analytical procedures. The mean of Total Ash values of all three batches vise; batch I, batch II and batch III of *Habb-i-shahtara* were found to be 0.1365±0.0012, 0.1374±0.0014 and 0.1370±0.0043 respectively and 0.0578±0.0550, 0.0236±0.0007 and 0.0266±0.0038 were the mean of acid insoluble ash value of all three batches batch I, batch II and batch III respectively. The mean values of Alcohol soluble extracts were found to be as for batch I-17.301±0.047654, batch II-17.2033±0.08745 and batch III-17.0886±0.0860 and mean values of all three batches of water-soluble extract of study formulation were found to be as 14.1173±0.0561, 14.0981±0.0973 and 14.2762±0.3327 for batch I, batch II and batch III respectively. Mean pH values of 1% aqueous suspension were found to be 5.12±0.00 and 5.13±0.00 and 5.15±0.00 whereas mean pH value of 10% aqueous suspension were found 5.14±0.00 and 5.15±0.00 and 5.15±0.00 for three batches. The mean values of average Loss of weight on drying at 105°C of *Habb-i-Shahtara* were found as 4.5044±0.0046, 4.5033±0.0094 and 4.5057±0.0060 and mean values of average weight of 20 pills were found to be as 539.6±16.33, 599.7±71.27 and 591.4±67.18. Mean values of disintegration time in aqueous media of all three batches were estimated as 89.666±0.577, 89±1.732 and 88.6666±1.527. The mean values of friability of study formulation were found to be 0.0193±0.002, 0.0751±0.005 and 0.0803±0.004 for batch I, batch II and batch III respectively. The mean percentage of hardness of study Unani formulation were found to be 10.6333±0.4163, 11.8666±1.7785 and 11.5733±1.1189. These findings are tabulated in Table 2.

Qualitative phytochemical analysis

In the present investigation the extracts of *Habb-i-Shahtara* were analyzed primarily for qualitative analysis in terms of alkaloids, tannins, terpenoids and saponins. Qualitatively it was observed that almost all extracts of unani formulation showed good contents of alkaloids, tannins, terpenoids and saponins and comparison made between each other. The result revealed the presence of medicinally active compounds in the formulation. The phytochemical characteristics of study Unani formulation were tested and summarized in the Table 3. The alcoholic extract of study formulation was shown the presence of alkaloids, carbohydrates, glycosides, saponins, proteins and tannins whereas the aqueous extracts of *Habb-i-Shahtara* shown the presence of alkaloid,

Table 3 — Qualitative Analysis of the Phytochemicals of *Habb-i-shahtara*

S. No.	Chemical Constituents	Observation and Results		
		Alcoholic Extract	Chloroform Extract	Aqueous Extract
1.	Alkaloid	+	-	++
2.	Carbohydrate	++	-	+++
3.	Glycosides	++	-	-
4.	Saponins	+	-	-
5.	Phenols	+	-	+
6.	Starch	-	-	-
7.	Proteins	++	-	-
8.	Steroids	-	+	-
9.	Tannins	+	-	+
10.	Flavonoids	-	-	+

Table 2 — Physicochemical Analysis of Unani formulation *Habb-i-Shahtara*

S. No	Physicochemical parameters	Mean Value ± SD Batch I	Mean Value ± SD Batch II	Mean Value ± SD Batch III
1	Total Ash values (% w/w)	0.1365±0.0012	0.1374±0.0014	0.1370±0.0043
2	Acid Insoluble Ash (% w/w)	0.0578±0.0550	0.0236±0.0007	0.0266±0.0038
3	Alcohol soluble extract (% w/w)	17.301±0.047654	17.2033±0.08745	17.0886±0.0860
4	Water soluble extract (% w/w)	14.1173±0.0561	14.0981±0.0973	14.2762±0.3327
5	pH of 01% aqueous suspension	5.12±0.00	5.13±0.00	5.15±0.00
6	pH of 10% aqueous suspension	5.14±0.00	5.15±0.00	5.14±0.00
7	Loss in wt. on drying at 105°C	4.5044±0.0046	4.5033±0.0094	4.5057±0.0060
8	Weight Variation of Habb	539.6±16.33	599.7±71.27	591.4±67.18
9	Disintegration Time	89.666±0.577	89±1.732	88.6666±1.527
10	Friability Test	0.0193±0.002	0.0751±0.005	0.0803±0.004
11	Hardness of Tablet	10.6333±0.4163	11.8666±1.7785	11.5733±1.1189

carbohydrates, tannins and flavonoids, while the presence of steroids was tested in the chloroform extract of study formulation depicted in Table 3.

HPTLC analysis of alcoholic extract of *Habb-i-Shahtara*

Alcoholic extract was spotted on silica gel "G" plate and developed with *toluene: ethyl acetate: methanol* (7:2:1, v/v/v) as mobile phase shows six major spots under UV 366 nm at R_f values 0.03 (red), 0.41 (pinkish blue), 0.50 (blue), 0.70 (pale yellow), 0.93 (light blue), 0.99 (red); and under UV 254 nm shows one spot at R_f values 0.03 (black); and under Iodine vapors shows two spots at R_f values 0.03, 0.99 (both brown) and under anisaldehyde sulphuric acid reagent and heating at 105°C shows five spots at R_f values 0.04 (light brown), 0.11 (grey), 0.27 (grey), 0.63 (grey), 0.99 (grey). The results are depicted in Table 4 and Figure 3 & 4.

Analysis of microbial load

The results of the microbial load were found within the permissible limit. No significant bacterial counts were found in *Habb-i-Shahtara*. The mean heterotrophic bacterial count of the herbal sample ranged from $48x10^2$ /g to $56x10^2$ /g (Unani formulation). Other microbes such as *Salmonella* spp, *Escherichia coli* and Total Yeast & Mould were not found and reveals that the total fungal propagule counts were absent Results are shown Table 5.

Table 4 — HPTLC of alcoholic extract of *Habb-i-Shahtara*

Peak list of Alcoholic extract of *Habb-i-Shahtara* at UV 366 nm

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	10.8	2241.39	96.63	927.37	0.02
2	38.9	8.85	0.38	6.27	0.40
3	54.4	3.94	0.17	2.96	0.61
4	61.7	3.90	0.17	2.33	0.71
5	68.2	5.09	0.22	3.33	0.80
6	82.0	56.46	2.43	23.39	0.99

Peak list of alcoholic extract of *Habb-i-Shahtara* at UV 254 nm

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	10.7	2354.62	70.75	1469.84	0.02
2	12.9	973.43	29.25	598.16	0.05

Peak list of alcoholic extract of *Habb-i-Shahtara* Upon exposure to Iodine vapour

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	10.7	725.86	59.78	423.08	0.02
2	12.9	463.69	38.19	288.85	0.05
3	82.6	24.65	2.03	19.46	0.99

Peak list of alcoholic extract of *Habb-i-Shahtara* upon derivatized with anisaldehyde sulphuric acid at 580 nm

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	10.9	2710.78	74.40	1248.28	0.03
2	18.2	145.80	4.00	118.22	0.12
3	29.7	65.76	1.80	36.92	0.28
4	53.3	83.40	2.29	27.87	0.60
5	63.9	217.10	5.96	40.31	0.74
6	82.6	420.74	11.55	274.94	0.99

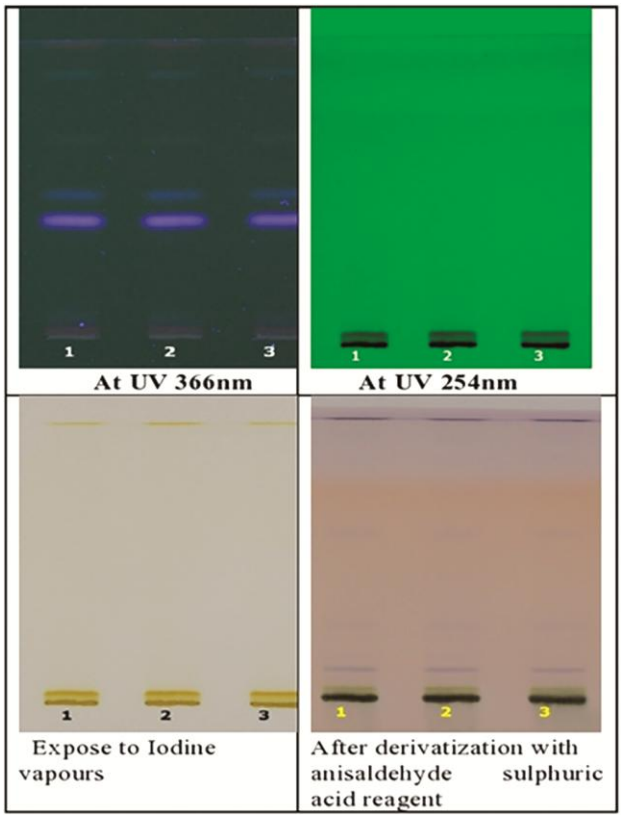

Fig. 3 — TLC of alcoholic extract of *Habb-i-Shahtara*

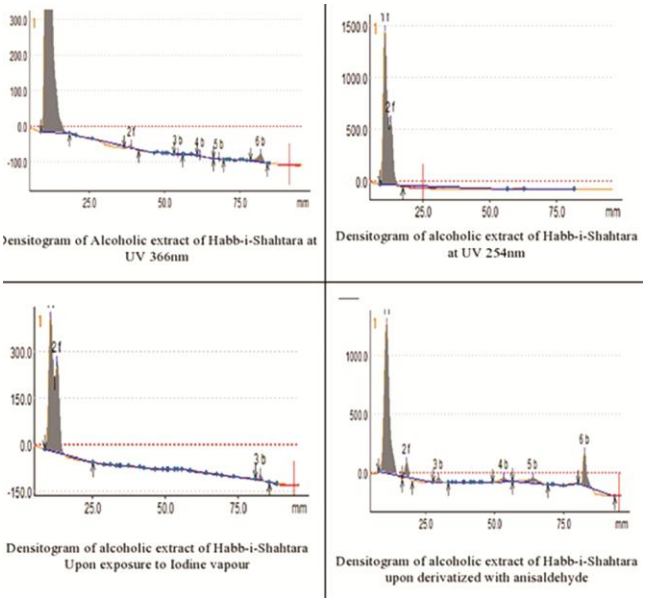

Fig. 4 — HPTLC of alcoholic extract of *Habb-i-Shahtara*

Table 5 — Safety Parameters of unani formulation *Habb-i-Shahtara*

S.No.	Parameter analyzed	Microbial Load determination Results			Permissible limits as per WHO
		Sample-I	Sample-II	Sample-III	
1.	Total *Bacterial* Load	$50x10^2$	$48x10^2$	$56x10^2$	Not more than 10^5 / g
2.	*Salmonella* Spp.	Nil	Nil	Nil	Nil
3.	*Escherichia. coli*	Nil	Nil	Nil	Nil
4.	Total *Fungal* count	Nil	Nil	Nil	Not more than 10^3 / g
		Aflatoxins determination			
S. No.	Parameter analyzed	Results			Permissible limits as per WHO
		Batch-I	Batch -II	Batch -III	
1.	B1	Nil	Nil	Nil	Not more than 0.50 ppm
2.	B2	Nil	Nil	Nil	Not more than 0.10 ppm
3.	G1	Nil	Nil	Nil	Not more than 0.50 ppm
4.	G2	Nil	Nil	Nil	Not more than 0.10 ppm
		Heavy metals determination			
S.No.	Parameters analyzed	Results			WHO Permissible Limits
1	Lead (Pb)	Not Detected			10 ppm
2	Cadmium (Cd)	Not Detected			0.3 ppm
3	Arsenic (As)	Not Detected			3.0 ppm
4	Mercury (Hg)	Not Detected			1.0 ppm

Estimation of aflatoxins

In this study the presence of aflatoxins such as B_1, B_2, G_1, and G_2 were not found in all the samples of *Habb-i-Shahtara*. Findings are tabulated in Table 5.

Heavy metals determination

The study formulation *Habb-i-Shahtara* was subjected to analysis of heavy metals such as lead, cadmium, arsenic and mercury. Results demonstrated that the heavy metals like lead, cadmium, arsenic and mercury were found below the detection limit depicted in Table 5.

Pesticides residues analysis

The study formulation *Habb-i-Shahtara* was analyzed by the Validate test method R-44 for 35 pesticide residues at Bureau Varitas India Testing Services Private Limited, Industrial Estate, Sanathnagar Hyderabad, 500018, Telangana. The test demonstrated that the pesticides in the study formulation were found below the limit of quantification.

Discussion

The world health organization has already make an attempt to progress, concern, understanding with respect to the traditional medicine quality and their uses to a large part of the population on this Earth[7]. In the past decades the global interest of trend for the improvement of interest in the indigenous system of medicine was increased. In regard of increasing public awareness in result of the good efficacy of the herbal drugs and the unwanted side effect of modern medicine, the requirement of the herbal Unani medicines increasing day by day on a large scale.

It is an accepted fact that any physiological active constituent of a medicinal plant and their products are responsible for a pharmacological action against a particular ailments[21]. This is may be due to synergetic effects or a catalytic effect. If the plant in devoid of active chemical compounds or that compound not present in optimum quantity the plant or such plant based formulations do not respond to the particular disease, therefore the quantitative standardization of drugs must be adopted and process of standardization should be established. In unani medicine several plant ingredients used together in the same compound formulation. That's why there should be quality control test for the entire preparation and for every single drug to ensure the better quality of the herbal medicine[22].

Several medicinal uses of *Habb-i-Shahtara* have been documented in the Unani literature and Unani pharmacopoeia. The results of present study showed that the presence active chemical constituents which are responsible to treat the imbalance occurs in *akhlāt* (*Humour*) and thus useful in the treatment of various skin diseases like *quba* (tinea), *ganj* was *a'fa* (alopacia), *jarab-wa-hikka* (scabies), etc.[9,10].

As the Organoleptic properties of drugs are the important parameter for the identification of crude as

well as compound formulations. *Habb-i-Shahtara* shows dark brown in color which is due to the presence of aelwa and saqmonia in its composition. The *Habb-i-Shahtara* having unpleasant odour which is due the presence of aelwa and pungent taste followed by bitter, the texture of habub (pills) were found a little hard with smooth surface. The polyherbalunani formulation *Habb-i-Shahtara* put through physicochemical analysis, which is very much supportive in establishing the standards along with the other parameters such as microscopic study, heavy metals analysis, microbial load and aflatoxins determination, and pesticide residue and these were found nil/or under the allowing permissible limits of WHO. HPTLC studies were thoroughly studied in alcohol extract. Phytochemical analysis was carried out for the determination of the presence of alkaloids, flavonoids, phenols, proteins, resins, saponins, sterols/ terpenes, sugar and tannins and in this way the phytochemical qualitative test for alkaloids, glycosides, carbohydrate, phenols, resins, proteins, steroids, saponins, tannins and flavonoids were found to be positive whereas starch and fixed oils were not found in *Habb-i-Shahtara*. Moor over the chemical constituents present in the study formulation may responsible for the effect of *dafi'tāfun* (anti-infective) which is useful in the treatment of various skin disease conditions. Thus the obtained data of the present study of *Habb-i-Shahtara* will provide help to maintain the consistency and bring quality assurance of that formulation and also helps in further development of standard parameters.

Conclusions

The results of present study reported that the polyherbal compound unani formulation *Habb-i-Shahtara* contains the medicinally beneficial phytoconstituents which provide the evidence in favors of ancient claims of this Unani formulation as recommended by the ancient Unani scholars to cure the various skin diseases. There are so many documented earlier studies which confirmed that the identified phytochemicals to be bioactive. Various researches revealed that the physiological and medicinal properties of herbal products are due to presence of these phytochemicals that's why they are used in different diseases. Therefore, the present results of study formulation *Habb-i-Shahtara* could be seen as a good source to develop the further scientific studies to be conducted in order to bring good clinical efficacy.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

Author's Contributions

UZ carried out the original research work in the laboratory experiments and wrote the manuscript. MHK and JIS conceptualize the study design and guide in performing the research and writing the manuscript. MARN help in carried out the HPTLC analysis and guide during the work and IA contributes during the laboratory experiment.

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