Standardization and HPTLC Fingerprinting of a Unani Compound Formulation Habb-e-Paan

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Abstract

With global realization that use of synthetic drugs is not safe on the long run, the medical fraternity at large is looking at alternatives from natural sources to combat diseases particularly those in which conventional modern system of medicine has little to offer. This realization on the one hand has increased demand for herbal drugs and on the other hand need for quality standardization of these drugs has gone up. Central Research Institute of Unani Medicine, Hyderabad being engaged in multidisciplinary research in Unani Medicine, working on standardization of herbal drugs used in this system of medicine. One such drug "Habb-e-Paan" which is prescribed in Unani system for Aatishak (Syphillis), Fasad-e-Dam (Putrefaction of Blood) has been taken up for standardization by modern techniques, so as to ascertain its quality. The parameters which were carried out are pharmacognostic studies, physico-chemical parameters, phytochemical screening, High performance thin layer chromatography, microbial load, aflatoxins, heavy metals, and pesticidal residues revealing specific identities for the particular drug and to evaluate pharmacopoeial standards. Results suggest that the drug is safe for therapeutic use and its batch to batch identification for quality control is possible on the basis of present study.

Keywords: Habb-e-Paan, Standardization, Physico-chemical analysis, HPTLC.

Introduction

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments (Sharma *et al*., 2008). The global resurgence of interest in herbal medicines has led to an increase in their demand leading to a decline in their quality, primarily due to a lack of adequate regulations pertaining to drugs (Rajini and Kanaki, 2008). WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and by applying suitable parameters and standards, In order to overcome certain inevitable shortcomings of the Pharmacopoeial monograph other quality control measures must be explored (Pifferi *et al*., 1999; Shinde, 2009; Singh and Soni, 2004; Street *et al*., 2008). Curative efficacies of compound herbal medicine are reliant on the quality and the quantity of the constituent single drugs as they contain specific bio-active marker species with specific pharmacological actions. Though, it is very difficult

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to identify the ingredients after the formulation is prepared and the organoleptic parameters like taste, odour, colour etc. will not establish the standard quality of the medicine.

A herbal formulation, Habb-e-Paan (Fig. 1) taken for the present study is a Unani compound formulation mentioned in National formulary of Unani medicine of India, Part-III, 1.41. The drug is prescribed in Unani system of medicine for Aatishak (Syphillis), Fasad-e-Dam (Putrefaction of Blood) and has its action as blood purifier. In order to standardize and to lay down the standard operating procedures (SOP's) and pharmacopoeial standards, the formulation was prepared in three batches at laboratory scale. It was subjected to analysis for microscopic study, physico-chemical parameters, microbial load, heavy metals, aflatoxins, pesticide residues and high performance thin layer chromatographic studies (Anonymous, 2009). The present paper describes the salient features of preparation, phytochemical screening, safety evaluation studies and High performance thin layer chromatographic studies for the drug.

Materials and Methods

Collection of material

Ingredients of formulation were procured from the pharmacy of Central Research Institute of Unani Medicine, Hyderabad, and identified with the help of a botanist. Arq-e-Paan has been prepared at in house laboratory by steam distillation method.

Preparation of the formulation (Bayaz-e-Kabir. II, pp.35).

It is prepared according to the composition of the formulation given in National Formulary which is as follows:

S. No	Name of the drug	Botanical Name	Part Used	Qty
1.	Sammul Far	White oxide of arsenic	Mineral	3g.
2.	Tabasheer	*Bambusa bambos* Druce	Crystals	3g.
3.	Kath Safaid	*Acacia leucophloea* Willd	Bark extract	3g.
4.	Arq-e-Paan	*Piper betle* Linn	Hydrodistillate.	Q.S.

Processing of raw material

Take all the ingredients of pharmacopoeial quality and clean all the ingredients. *Summul far* (Sankhiya) fine powder is immersed in sufficient quantity of fresh *Aab-e-Leemu* (Lemon juice) and ground in a mortar of china clay or glass till

the juice is completely absorbed. This process is repeated seven times to obtain *Summul far* or *Sankhiya mudabbar*. Ground the powder of each drug separately to obtain fine powder and pass through 80 mesh sieve. Mix the fine powders thoroughly with Arq-e-pan and prepare Huboob by mechanical process. Store the huboob so obtained in dry containers to protect from light and moisture.

Preparation of the Tablets

The tablets were prepared as per the procedure described by Bayaz-e-Kabir. II, p.35. The granules were made into 500mg tablets (excluding binding material weight) using rotary tablet punching machine (Cadmach-GMP model).

Chemical analysis

Physico-Chemical parameters of the prepared compound formulation Habb-e-Paan were studied such as total ash, acid insoluble ash, water soluble ash, solubility matter in alcohol and water, loss on drying at 105^0C, microbial load, aflatoxins, pesticide residue and GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used to determine the concentration of heavy metals as per the methods described in WHO guidelines (Anonymous, 1998). Phytochemical screening was carried out in different solvents extracts such as petroleum ether, Chloroform, Ethyl acetate, methanol, Ethanol, and aqueous as per the methods described by Trease and Evans (1972).

HPTLC analysis

DESAGA Sarstedt Gruppe system is used for analysis along with Automatic TLC applicator and UV visible cabinet as imaging system, the instrument had Proquant 1.6 version as software system for documentation.

Preparation of Extract of the drug for HPTLC analysis

Five grams fine powder of Habb-e-Paan was reflux on water bath for 30 min in different solvent separately through sohxlet. Later the contents were removed and filtered through Whattmann No. 41 filter paper and evaporated the solution to 20 ml. Thus the solution so obtained was used as sample for the determination of components.

Development and determination of the solvent system

Sample Applied	:	Sample drug solution of about 10µl.
Solvent system	:	Toluene: Ethyl acetate: Methanol (5: 4: 1)

The sample was spotted with the help of Automatic TLC applicator system of the DESAGA Sarstedt Gruppe on Precoated Aluminium Sheets of Silica Gel 60 F_{254} (Merck) After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated above is selected in its proportional ratio and developed in the Twin through chamber of TLC to the maximum height of the plate so that it can be able to separate the components on the polar phase of silica gel and that of mobile phase of solvent system. The formulation and its ingredients were spotted separately and developed the TLC plate.

Development of HPTLC technique

After developing, TLC plates were dried completely and detected with the suitable detection system like UV Cabinet system for detection of spots at 254, 366nm and also under iodine vapours and after derivatizing with anisaldehyde sulphuric acid reagent as shown in the figure 3. Further it was scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 254nm, 366nm, 580nm and the overlay densitogram at 254nm and also Densitogram of ethyl acetate extract at 254nm as shown in the figure 4 in which peaks appeared 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. The separation of the components in the compound formulation and its ingredients are corresponding compared with respect to Rf values.

Table 1 : Physico-chemical parameters of the compound formulation 'Habb-e-Paan'.

S. No	Parameters	Sample I	Sample II	Sample III
1	Ash values Total Ash (%)	31.38-31.48	31.63-31.74	31.70-31.74
	Acid insoluble ash (%)	26.38-26.65	26.80-26.83	26.95-27.00
2	Alcohol Soluble matter (% w/w)	6.93-7.34	7.87-7.89	7.69-7.75
3	Water soluble matter (% w/w)	12.56-12.65	12.72-12.73	12.72-12.81
4	PH of 1% aq. Solution	4.13	4.12	4.10
	PH of 10% aq. Solution	3.58	3.57	3.58
5	Disintegration time in min.	12	12	12
6	Loss on drying at 105°C (%w/w)	5.29-5.31	5.33-5.36	5.43-5.58

Table 2 : Phytochemical screening of the nature of compounds present in different solvent extracts of *Habb-e-Paan*.

S. No.	Phyto constituent	Pet. ether ext.	$CHCl_3$ ext.	E.A. ext.	Meth ext.	ethanol ext.	Aqueous ext.
1.	Alkaloid	-	-	-	+	+	+
2.	Carbohydrates	-	-	+	+	+	+
3.	Glycosides	-	-	-	-	-	-
4.	Phenols	-	-	+	++	++	++
5.	Steroids	-	+	++	++	++	++
6.	Tannins	-	-	+	++	++	++
7.	Flavonoids	-	-	-	-	-	-
8.	Saponins	-	-	-	-	-	-
9.	Starch	-	-	-	-	-	-

Table 3 : Peak list of densitogram of the Solvent extracts of Habb-e-Paan at UV 254nm.

Peak no	Sammul Far	Katha	Tabasheer	Arq-e-paan	PE ext	$CHCl_3$ ext	EA ext	MeOH ext	Aq. ext
1	-	0.04	-	0.03	0.03	0.03	0.03	0.03	0.03
2	-	-	-	-	-	-	0.20	-	-
3	-	0.35	-	-	-	-	0.34	0.34	0.32
4	-	-	-	-	0.67	0.64	0.67	0.64	0.61
5	-	-	-	-	0.80	0.77	0.79	0.82	-
6	0.95	-	0.94	0.93	0.91	0.94	0.96	0.93	0.94

Table 4 : Peak list of densitogram of the Solvent extracts of Habb-e-Paan at UV 366 nm.

Peak no	Sammul Far	Katha	Tabasheer	Arq-e-paan	PE ext	$CHCl_3$ ext	EA ext	MeOH ext	Aq. ext
1	0.01	0.02	-	0.02	0.02	0.02	0.02	0.02	0.02
2	-	-	-	-	-	-	0.09	0.09	-
3	-	-	-	-	-	-	0.12	0.11	-
4	-	0.35	-	-	-	-	0.35	0.35	-
5	-	-	-	-	-	-	0.69	0.67	-
6	-	-	-	-	-	0.74	0.75	0.76	-

Peak no	Sammul Far	Katha	Tabasheer	Arq-e-paan	PE ext	$CHCl_3$ ext	EA ext	MeOH ext	Aq. ext
7	-	-	-	-	0.80	0.80	0.80	0.82	-
8	-	0.94	-	-	0.92	0.93	-	-	-
9	-	-	0.95	0.98	-	0.95	0.97	-	0.97

Table 5 : Peak list of densitogram of the Solvent extracts of Habb-e-Paan at 580nm under iodine vapour detection.

Peak no	Sammul Far	Katha	Tabasheer	Arq-e-paan	PE ext	$CHCl_3$ ext	EA ext	MeOH ext	Aq. ext
1	-	0.02	-	-	-	0.03	0.03	0.03	0.03
2	0.34	0.34	-	-	-	0.34	0.34	0.35	0.34
3	-	-	-	-	-	-	0.42	-	-
4	-	0.49	0.49	-	-	-	0.49	0.49	0.50
5	-	-	-	-	-	-	0.57	0.57	-
6	-	-	-	0.77	0.77	0.77	0.77	0.78	-
7	-	-	-	-	0.84	-	-	-	-

Table 6 : Peak list of densitogram of the Solvent extracts of Habb-e-Paan after derivatized with anisaldehyde sulphuric acid reagent at 580 nm.

Peak no	Sammul Far	Katha	Tabasheer	Arq-e-paan	PE ext	$CHCl_3$ ext	EA ext	MeOH ext	Aq. ext
1	-	0.02	-	-	-	0.03	0.03	0.02	0.02
2	-	0.34	-	-	-	-	0.34	0.35	-
3	-	0.49	-	-	-	-	0.49	0.49	0.50
4	-	-	-	-	0.77	0.77	0.77	0.78	-

Table 7 : Microbial Contamination

S. No	Parameter analyzed	Results	Permissible limits as per WHO
1	Total *Bacterial* Load	39×10^3	Not more than 10^5 / g
2	*Salmonella Spp*.	Nil	Nil
3	Escherichia. Coli	Nil	Nil
4	Total *Fungal* count	1×10^2	Not more than 10^3/g

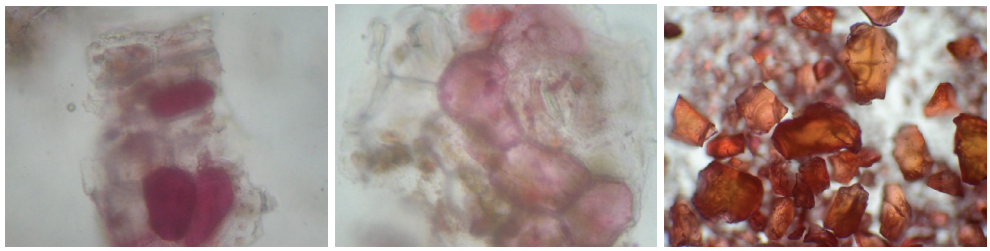
Table 8 : Aflatoxin Contamination

S. No	Parameter analyzed	Results	Permissible limits as per WHO
1	B1	Nil	Not more than 0.50 ppm
2	B2	Nil	Not more than 0.10 ppm
3	G1	Nil	Not more than 0.50 ppm
4	G2	Nil	Not more than 0.10 ppm

Table 9 : Heavy Metal Analysis

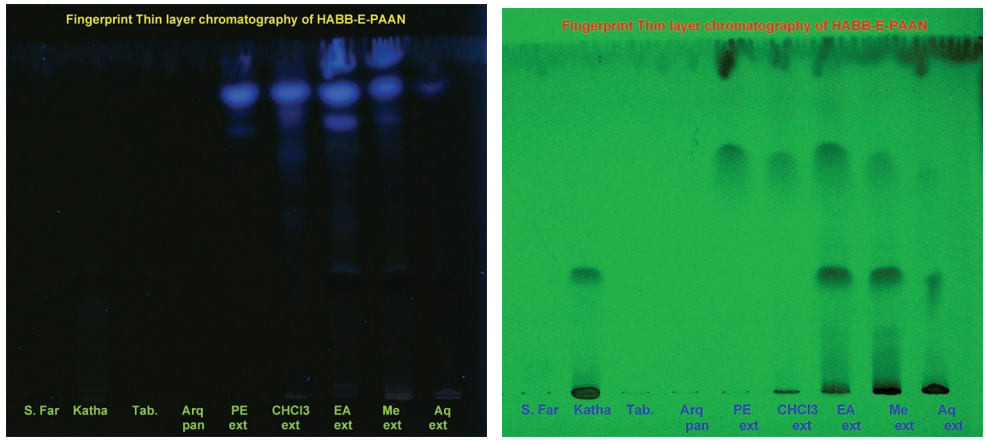
S. No	Parameter analyzed	Results	Permissible limits as per WHO
1	Arsenic	Nil	Not more than 3.0 ppm
2	Cadmium	Nil	Not more than 0.3 ppm
3	Lead	Nil	Not more than 10.0 ppm
4	Mercury	Nil	Not more than 1.0 ppm

Fig. 1: Finished Formulation Habb-e-Paan.

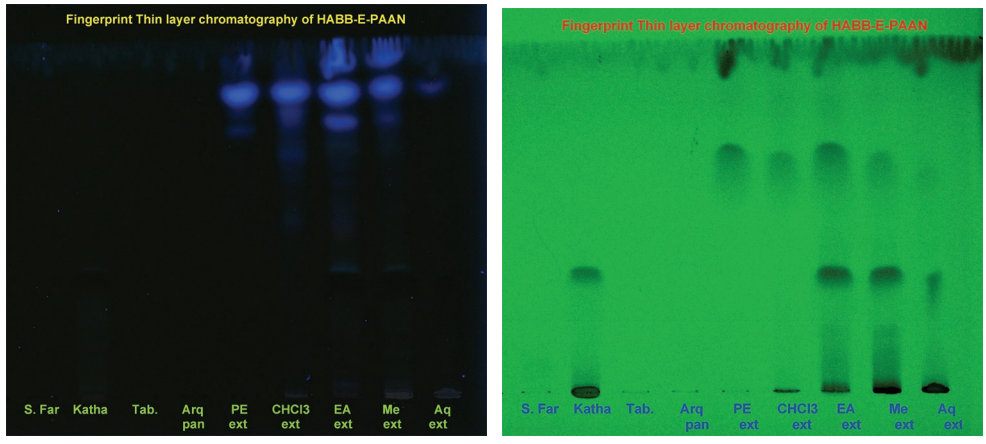


Parenchyma cells with reddish tannin content | Sac like Parenchymatous cells | Abundant Resin content

Fig. 2: Powder microscopic properties of the formulation Habb-e-Paan



A) At UV 366 nm | B) At UV 254 nm



C) Exposed to I_2 vapours | D) Derivatized with anisaldehyde sulphuric acid reagent.

Fig. 3. TLC plates of different solvent extracts of Habb-e-Paan and its ingredients Summul far, Katha, Tabasheer, Arq-e-paan A) At UV 366 nm, B) At UV 254 nm, C) Under Iodine vapours D) At visible region after derivatizing with Anisaldehyde sulphuric acid reagent.

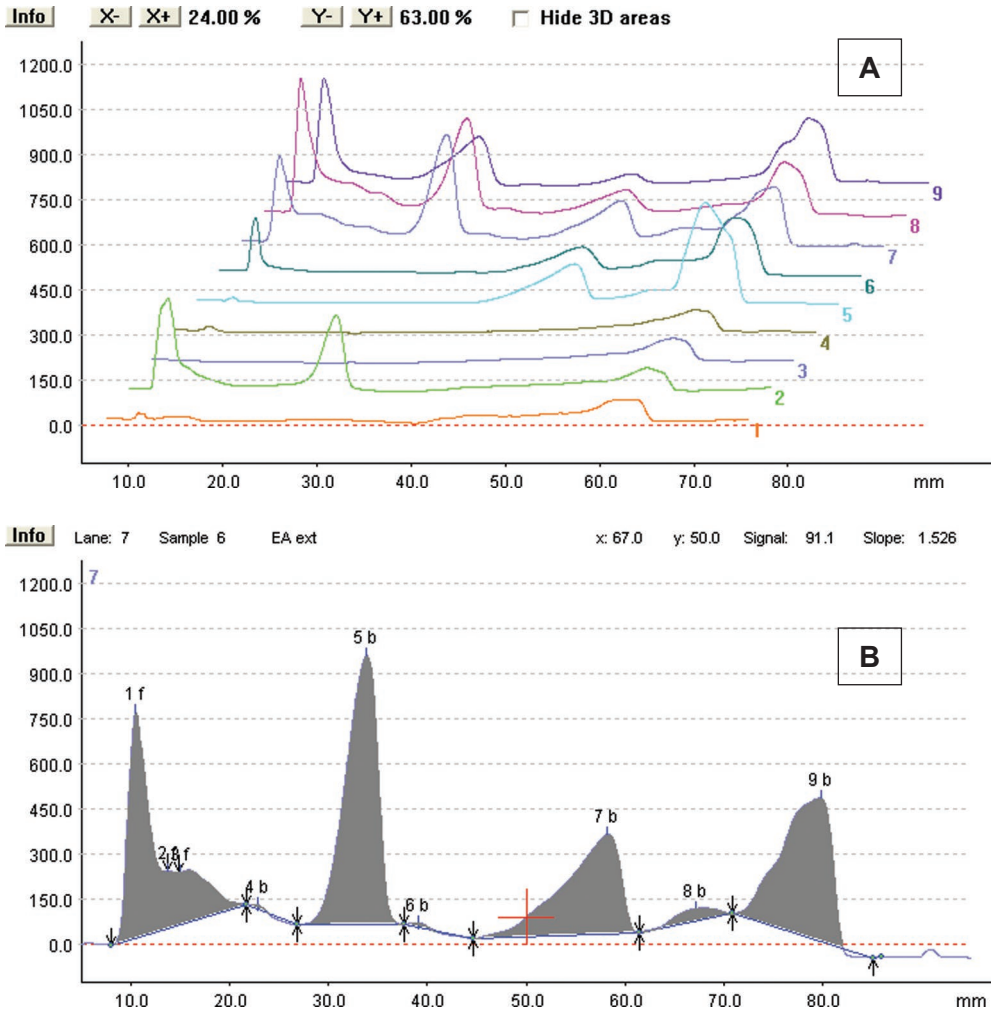


Fig. 4. A) Overlay Densitogram of Habb-e-Paan and its ingredients as spotted on TLC plate (1-9), B) Densitogram of Habb-e-Paan ethyl acetate extract at 254 nm.

Results and Discussion

Analytical Profile

Organoleptic Characters

Light brown coloured Unani pills with white spots pleasant smell and sweet taste.

Identification

Powder Microscopy

Take fine powder of six tablets and immersed in the water for half an hour. Material was stirred with a glass rod and supernatant was discarded. Residue

was taken on glass slide, treated with iodine solution, Safranin and mounted with glycerine. The prepared slide was subjected for microscopical studies. Microscopical observations clearly showed the presence of abundant resin cells, scarcely starch grains, reddish tannin content and sac like parenchymatous cells as shown in figure 2.

Physico-Chemical Standards

The Physico-Chemical Parameters data as given in table 1 is expressed as mean values of the three readings calculated. Total ash was found to be 31.38-31.74, and acid insoluble ash 26.38-27.00gm%; whereas Alcohol soluble matter in terms of %w/w is found to be 6.93-7.89 and water soluble matter as 12.56-12.81; The moisture content i.e., Loss of weight on drying at 105^0C found to be 5.29-5.58 gm%. P^H of the 1% aqueous solution observed as 4.10-4.13 and 10% aqueous solution observed as 3.57-3.58; and Disintegration time of tablet was 12min. Phytochemical screening for the phytoconstituents were carried out and are represented in the table 2. The results of total bacterial load and total fungal count of the microbial studies were within the permissible limits and the other parameters were found to be absent in the drug. The analysis of aflatoxins and heavy metal analysis showed that the drug was free from any contaminations. These findings as observed for microbial load, aflatoxin contamination and heavy metal analysis are given in tables 7, 8 and 9 respectively.

HPTLC Analysis

HPTLC fingerprint studies of methanolic extract of Habb-e-Paan along with its ingredients was carried out and TLC plate developed and detected using the UV visible chamber which clearly showed various spots at UV 254nm and 366nm in the densitogram and also under iodine vapours and after derivatizing with anisaldehyde sulphuric acid reagent. The corresponding Rf values under each detection is illustrated in the tables 3,4,5 and 6. The corresponding R_f values of the compound formulation in different extracts where coinciding with corresponding position of spot with the ingredients and its Rf values. Detection under 254nm Rf values of Sammul Far (0.95), Katha (0.04,0.35), Tabasheer (0.94) and Arq-e-paan (0.03,0.93) are correspondingly coinciding with the formulations Rf values indicating the presence of constituents form the ingredients. Similary at UV 366nm. Upon exposure of TLC plate to Iodine vapours shows Rf values of Sammul Far (0.34), Katha (0.02, 0.34, 0.49), Tabasheer (0.49) and Arq-e-paan (0.77) are correspondingly coinciding with the formulations Rf values indicating the presence of constituents

form the ingredients. The same in respect of detection after derivatizing with anisaldehyde sulphuric acid reagent. Thus the established HPTLC fingerprinting profile helps to authenticate the formulation in batch to batch consistency and quality control analysis of formulation as a reference.

Conclusion

The drug under study was subjected to Physico-chemical analysis, which is helpful in establishing the standard along with the other parameters such as phytochemical screening, microscopic study, HPTLC analysis. Safety evaluation of drug such as Heavy metal analysis, aflatoxins contamination analysis was done and found absent; microbial load was found within the permissible limits of WHO guidelines. Modern technique of HPTLC analysis was employed in respect to standardization and to separate the compounds which can be isolated for further studies. Consequently, the drug was brought up in determining and ascertaining its quality standard**.** The study is likely to help in the quality assurance of drug used in the Unani System of Medicine and in development of standard parameters. The development of this traditional system of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural herbal products in the healthcare.

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