Standardization of a Unani Drug 'Habb-e-Banafsha'

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Abstract

Traditional medicines are being used extensively all over the world for primary health care. Nowadays, the needy mass requires safe and efficacious herbal medicines due to their lesser side effects. Hence there is an urgent need for standardization and development of standard operative procedures (SOPs) of traditional medicine to provide the quality medicines in the market. To ensure the genuineness and quality of drug, work on evaluation of pharmacopoeial standards was carried out on Habb-e-Banafsha which is used to cure Asthma, Bronchitis & Caphalagra. The Standard Operative Procedure (SOP) for the preparation of quality medicine was developed at the laboratory scale, using botanically identified and standardized ingredients. The samples of the formulation were subjected to evaluate the pharmacopoeial parameters such as Microscopy, Physico-chemical analysis, TLC, HPTLC and WHO parameters viz. microbial contamination, pesticide residues, aflatoxins level and the presence of heavy metals to ascertain the quality of the drug.

Keywords: SOP, Microscopy, TLC, HPTLC, UV, Heavy metals.

Introduction

Habb-e-Banafsha is a Unani poly-herbal formulation, categorized under Huboob in the National Formulary of Unani Medicine, Part III (Anonymous, 2001). Habb-e-Banafsha is reputed for its expectorant (Munaffis-e-Balgham), sedative (Musakkin), Coctive, concoctive and Maturative (Munzij) action and is used in the treatment of Suda (Cephalagia), sual (Bronchitis) and Zeeq-un-Nafas (Asthma) (Kabiruddin, 1938).

The present study was aimed to standardize this important Unani drug in order to ensure its authenticity, quality and efficacy. Habb-e-Banafsha was prepared according to the formula as described in NFUM-III using four ingredients of plant origin (UPI, Part I, Vol. I, II, V) (Table 1). In order to lay down SOPs and pharmacopoeial standards, this drug was prepared in laboratory at DSRI, Ghaziabad. The present paper describes the salient features of preparation, microscopical characters, physico-chemical parameters, TLC, HPTLC, UV spectroscopy, heavy metals estimations, Aflatoxins and pesticide estimation (Anonymous, 2000) not reported so far.

Materials and Methods

Preparation of Drug:

All the ingredients were procured from local raw drug dealer and were identified

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Table 1: Formulation Composition

S.No.	Ingredients	Botanical Name	Part used
1.	Banafsha	*Viola odorota* L.	Flower
2.	Turbud	*Operculina turpethum* (L.) S. Manso	Root
3.	Rubb-us-Soos	*Glycyrrhiza glabra* L.	Root
4.	Arq-e-Barg-e-Kasni Sabz	*Chichorium intybus* L.	Fresh leaf juice

botanically (Wallis, 1967; Trease & Evans, 1972) using pharmacognostical methods. The ingredients were further validated by comparing with a monograph available in UPI (Part I), Vol. I, II & V.

All the ingredients were taken of pharmacopoeial quality. The ingredients were cleaned and dried under shade to remove the moisture if any. The ingredients (S. No. 1-3, Table 1) were crushed separately in an iron-mortar to obtain their coarse powders. The coarse powders were further ground in a mixer grinder to get the fine forms. The fine powders were mixed thoroughly and sieved through mesh No. 80. Arq-e-Barg-e-Kasni sabz was then added to the mixture and again mixed thoroughly to obtain the lubdi mass. Huboob was prepared from the lubdi mass by mechanical process and dried under shade. The prepared Huboob were stored in tightly closed glass container free from moisture and kept in a cool and dry place.

Microscopy

5 g of the powdered drug was taken and stirred gently with hot water in a beaker. The supernatant was discarded and the residue was washed with the distilled water. A little residue was stained with Iodine solution and mounted in 50% glycerine. Some of the residue was heated in chloral hydrate solution and mounted in 50% glycerine and a little residue was boiled in 2% potassium hydroxide solution, washed with distilled water and mounted in 50% glycerine (Johansen, 1940; Wallis, 1967).

Chemical analysis

Physico-chemical parameters of Habb-e-Banafsha such as removal of foreign matters, solubility in water, ethanol and petroleum ether (60-80°), total ash, acid insoluble ash and water soluble ash, loss on drying at 105°C, pH values of 1% and 10% aqueous solution and volatile oil estimation were analysed by standard methods (Anonymous, 1987; 1998). Detection of microbial load, Aflatoxins and analysis of pesticide residue (Anonymous, 2000) and heavy metal were carried out as per standard methods (Sahito *et al*., 2001).

Preparation of extract for TLC/HPTLC

Samples of all the three batches of the formulation were extracted separately with chloroform and ethanol. The extracts were concentrated and made up to 10ml in a volumetric flask separately. These solutions were used for the TLC/ HPTLC finger print analysis by employing CAMAG Linomat IV sample applicator on aluminium TLC plate pre-coated with silica gel 60 F_{254} (E. Merck). The chromatograms were developed using the solvent system toluene: ethyl acetate in the ratio 9:1 & 1:1 respectively for chloroform and ethanol extracts. The plates were dried at room temperature and observed the spots at UV-254 and UV-366. Further the plates were dipped in 1% vanillin-sulphuric acid reagent and heated at 105^0 C till coloured spots appeared. (Wagner *et al*., 1984; Sethi, 1996; Stahl, 1996)

Preparation of extract for U.V. Spectroscopic studies

1g of the drug was extracted with 100 ml. Petroleum ether (60-80º) by refluxing for 15 minutes on water bath and filtered. The solution was made up to 100 ml. in volumetric flask. This solution was used for U.V. analysis and pure petroleum ether (60-80º) was used as blank solution (Willard *et. al*, 1965).

Observations

Habb-e-Banafsha is small brown pill having hard texture, characteristic odour and slightly sweetish taste. The drug did not show any filth, fungus or objectionable matter while the sample was spread in a Petri dish (Fig. 1).

The microscopy shows xylem vessels with pitted thickenings, cork cells, few crystal sheath of parenchymatous cells containing a prism of calcium oxalate (Rubb-us-soos), parenchyma having rosettes of prismatic calcium oxalate crystals uni and biseriate medullary rays, resin cells, tubular and polygonal epidermal cells, cellulosic fibres with pointed tips (Turbud). Thin walled trichomes with printed tips arising from epidermal cells with radially oriented subsidiary cells. Vessels narrow, seleriform, tailed at both end, round shaped pollen grains (Banafsha).

The results observed for the physico-chemical data, microbial load, aflatoxins, pesticide residues, heavy metals, TLC and HPTLC finger printing are shown in Table 2,3,4,5,6,&7 respectively.

Results and Discussion

The physico-chemical data of the drug are shown in table-2. The low value of water soluble extractive (15.80-17.04%) shows the absence of any inorganic

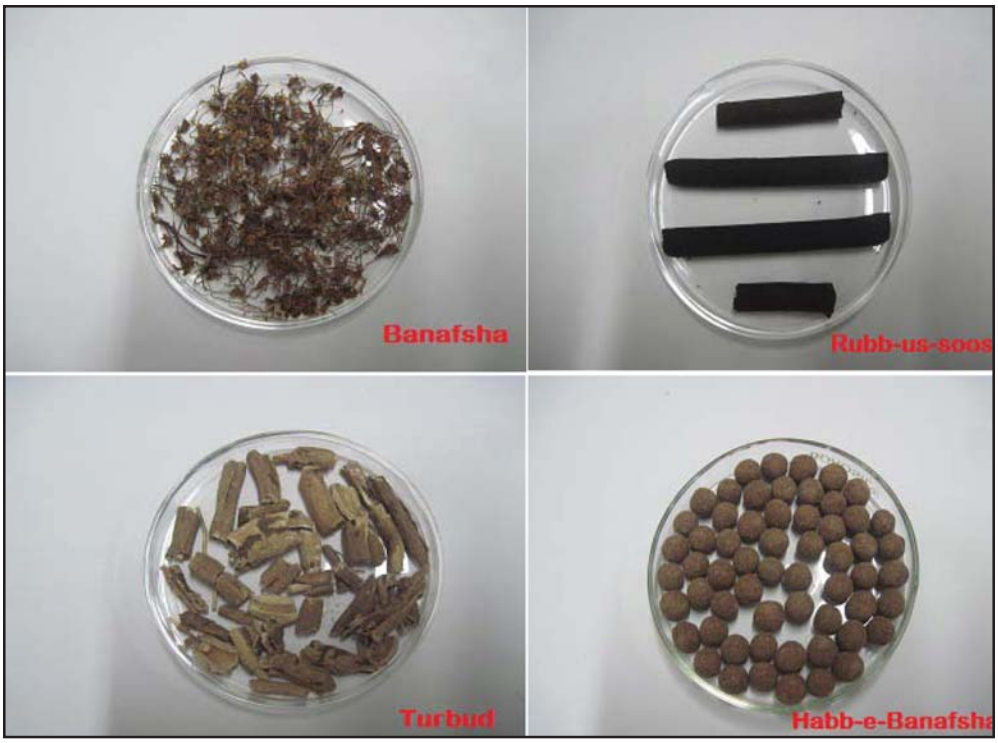


Fig. 1 : Unani drug Habb-e-Banafsha

Table2: Physico-chemical Parameters

S.No.	Parameters	Results
1.	Water solubility	15.80 - 17.04 %
2.	Alcohol solubility	3.56 - 4.92 %
3.	Pet ether (60^0-80^0) solubility	2.52 - 3.86 %
4.	Loss in weight on drying at 105^0 C	8.40 - 9.48 %
5.	Total Ash	10.36 - 11.75 %
6.	Acid Insoluble Ash	2.36 - 2.98 %
7.	Water Soluble Ash	6.52 - 7.14 %
8.	pH of 1% Solution	5.14 - 5.86
9.	pH of 10 % Solution	4.56 - 4.84
10.	Volatile Oil	Traces

constituents. The moisture content in the drug was also low as the loss in weight on drying at 105^0C occurred below 10%. The low value of acid insoluble ash of the drug indicates that the drug is free from siliceous matter. The results of microbial studies viz. TBC and TFC are within the permissible limits while the other microbes are absent (Table-3). The results of Aflatoxins studies (Table-4) and pesticide residues (Table-5) show that the drug is free from Aflatoxins as

Table 3: Microbial Load

S.No.	Parameter Analyzed	Results	Permissible limit as per WHO
1.	Total Bacterial load	$2x10^3$ cfu/g	10^5 cfu/g
2.	Total fungal count	< 10 cfu/g	10^3 cfu/g
3.	Enter obacteriaceae	Absent	Nil
4.	Escherichia coli	Absent	Nil
5.	Salmonella cpp.	Absent	Nil
6.	Staphoilococcus aureus	Absent	Nil

Table 4: Aflatoxins Level

S.No.	Parameter Analyzed	Results	Detection limits
1.	B-1	Not detected	0.50 ppm
2.	B-2	Not detected	0.10 ppm
3.	G-1	Not detected	0.50 ppm
4.	G-2	Not detected	0.10 ppm

Table 5: Pesticide Residue

S.No.	Parameter Analyzed	Results	Limit
1.	Chlorpyriphos	Not detected	0.20 mg/Kg
2.	DDT	Not detected	1.00 mg/Kg
3.	Endosulfan	Not detected	3.00 mg/Kg
4.	Malathon	Not detected	1.00 mg/Kg
5.	Parathion	Not detected	0.50 mg/Kg

Table 6: Heavy Metals

S.No.	Heavy Metal Analyzed	Results	Permissible limit as per WHO
1.	Arsenic	Not detected	3.00 ppm
2.	Cadmium	Not detected	0.30 ppm
3.	Mercury	Not detected	01.00 ppm
4.	Lead	Not detected	10.00 ppm

well as pesticide residues. The content of heavy metals are below detection limits (Table-6) suggests that drug is free from any type of heavy metal contamination.

HPTLC profile

HPTLC methods are reliable and convenient means in identification of crude drugs as well as compound formulations as each plant species produces a distinct chromatogram. TLC of all the three batches of Habb-e-Banafsha were observed under UV 254nm, UV 366nm and after derivatization (Table-7). Chromatogram of chloroform extract shows 08 spots under UV 254nm (Fig. 2), 12 spots under UV 366nm (Fig. 3) and 09 spots after derivatization (Fig. 4). The finger print of chloroform extract shows 13 peaks out of which peaks at R_f 0.09, 0.61, 0.70 and 0.77 were major peaks whereas peaks at R_f 0.04, 0.17, 0.39, 0.42, 0.49, 0.55, 0.85, 0.92 and 0.96 are relatively smaller peaks (Fig. 5). HPTLC Chromatogram of chloroform extract is shown in Fig. 6.

Table 7: TLC Results

S. No.	Extract	Solvent System	Developing reagent	Rf Values with colour		
				UV254nm	UV366nm	After derivatisation
1.	Chloroform	Toluene :	Vanillin –	0.95 Black	0.95 Red	0.91 Grey
		Ethyl	Sulphuric	0.66 Black	0.91 Red	0.83 Grey
		acetate (9:1)	acid	0.60 Black	0.67 Red	0.66 Green
				0.53 Black	0.60 Red	0.60 Yellowish green
				0.47 Black	0.55 Blue	0.53 Violet
				0.42 Black	0.52 Red	0.40 Violet
				0.33 Black	0.47 Red	0.31 Grey
				0.10 Black	0.31 Red	0.14 Violet
					0.26 Red	0.10 Grey
					0.18 Red	
					0.13 Red	
					0.11 Blue	
2.	Ethanol	Toluene :	Vanillin –	0.95 Black	0.95 Red	0.85 Violet
		Ethyl	Sulphuric	0.89 Black	0.87 Pink	0.74 Violet
		acetate (1:1)	acid	0.74 Black	0.74 Pink	0.71 Grey
				0.69 Black	0.71 Red	0.58 Yellow
				0.58 Black	0.61 Brown	0.46 Grey
				0.45 Black	0.51 Red	0.39 Grey
				0.30 Black	0.43 Violet	0.35 Violet
				0.14 Black	0.35 Red	0.14 Yellowish green
				0.11 Black	0.19 Violet	0.11 Grey
					0.11 Red	

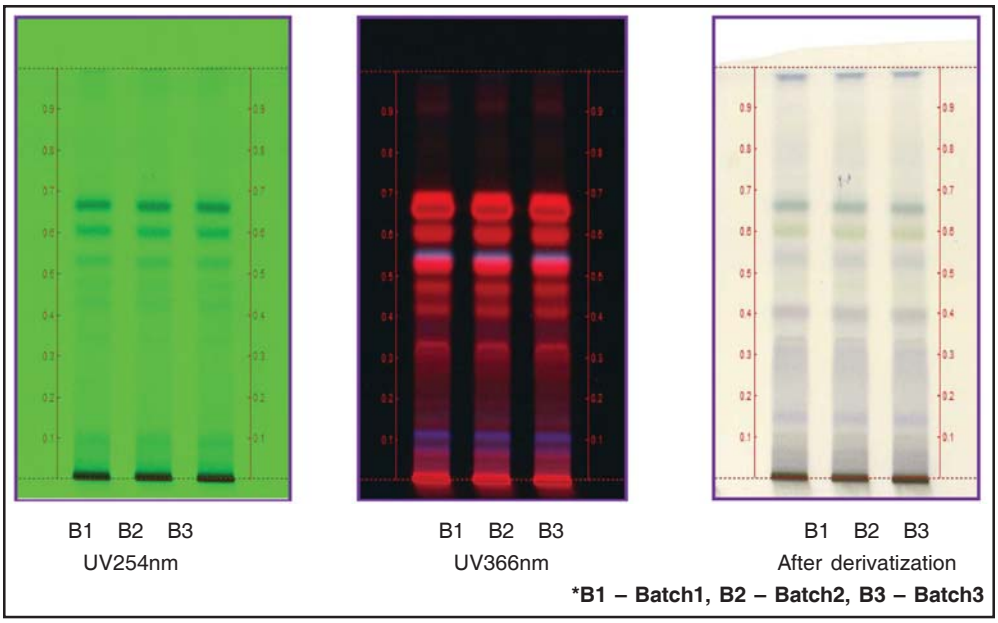


Fig. 2, 3 4: TLC of Chloroform extract of Habb-e-Banafsha

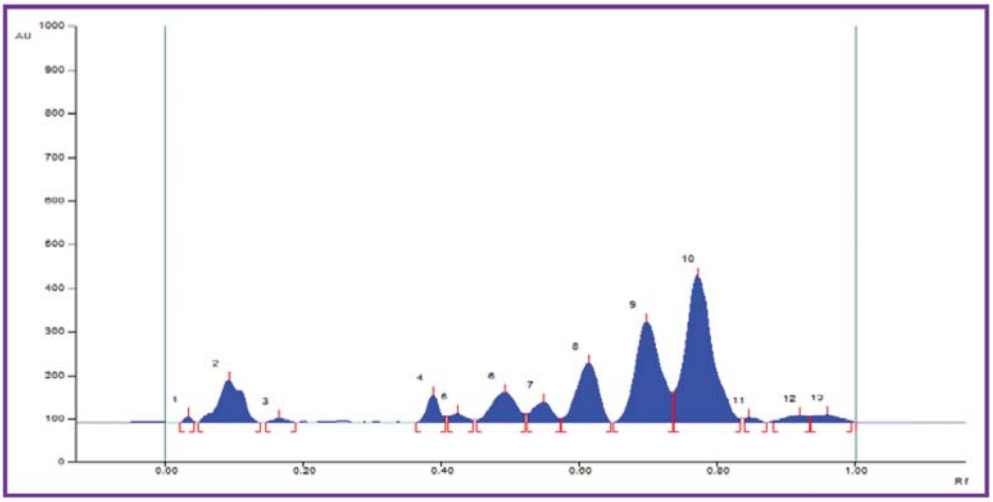


Fig. 5: HPTLC Finger printing of Chloroform extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.04 Rf	13.7 AU	1.28 %	0.04 Rf	1.1 AU	100.5 AU	0.39 %
2	0.05 Rf	0.1 AU	0.09 Rf	96.8 AU	9.02 %	0.14 Rf	0.2 AU	2393.8 AU	9.36 %
3	0.15 Rf	0.3 AU	0.17 Rf	10.6 AU	0.99 %	0.19 Rf	0.5 AU	145.0 AU	0.57 %
4	0.36 Rf	0.7 AU	0.39 Rf	63.6 AU	5.93 %	0.41 Rf	14.0 AU	783.8 AU	3.06 %
5	0.41 Rf	14.9 AU	0.42 Rf	20.0 AU	1.86 %	0.45 Rf	5.9 AU	324.3 AU	1.27 %
6	0.45 Rf	5.8 AU	0.49 Rf	69.1 AU	6.44 %	0.52 Rf	21.3 AU	1779.6 AU	6.96 %
7	0.53 Rf	20.3 AU	0.55 Rf	47.1 AU	4.39 %	0.57 Rf	9.5 AU	956.8 AU	3.74 %
8	0.58 Rf	9.8 AU	0.61 Rf	136.8 AU	12.75 %	0.65 Rf	0.6 AU	2954.6 AU	11.55 %
9	0.65 Rf	0.3 AU	0.70 Rf	231.6 AU	21.59 %	0.74 Rf	70.2 AU	6085.9 AU	23.79 %
10	0.74 Rf	71.1 AU	0.77 Rf	336.4 AU	31.36 %	0.83 Rf	9.6 AU	9026.0 AU	35.28 %
11	0.84 Rf	9.6 AU	0.85 Rf	12.1 AU	1.12 %	0.87 Rf	0.1 AU	154.2 AU	0.60 %
12	0.88 Rf	3.3 AU	0.92 Rf	16.6 AU	1.55 %	0.94 Rf	14.0 AU	406.8 AU	1.59 %
13	0.94 Rf	14.5 AU	0.96 Rf	18.5 AU	1.72 %	1.00 Rf	3.0 AU	470.5 AU	1.84 %

Fig. 5: R_f Values of HPTLC Finger prints of Chloroform extract

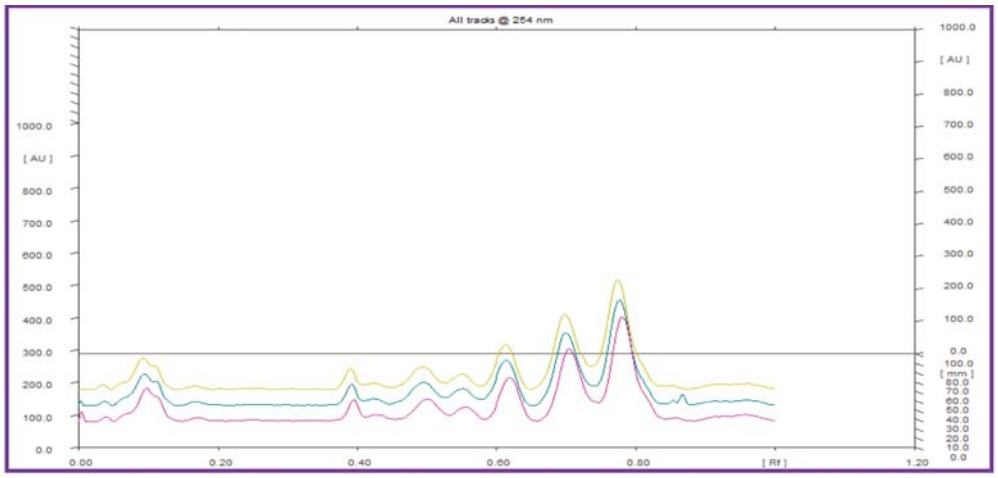


Fig. 6: HPTLC Chromatogram of Chloroform extract

Similarly TLC of ethanol extract shows 09 spots under UV 254nm (Fig. 7), 10 spots under UV 366nm (Fig. 8) and 09 spots after derivatization (Fig. 9). The finger print of ethanol extract shows 13 peaks out of which peaks at R_f 0.03, 0.07, 0.17, 0.34, 0.53, 0.68 and 0.87 were major peaks whereas peaks at R_f 0.22, 0.40, 0.45, 0.59, 0.78 and 0.82 are relatively smaller peaks (Fig. 10). HPTLC Chromatogram of ethanol extract is shown in Fig. 11.

The HPTLC densitometry chromatograms of chloroform and ethanol extract of all the three batches were found to be similar when scanned at 254nm. It indicates batch to batch consistency of the compound formulation.

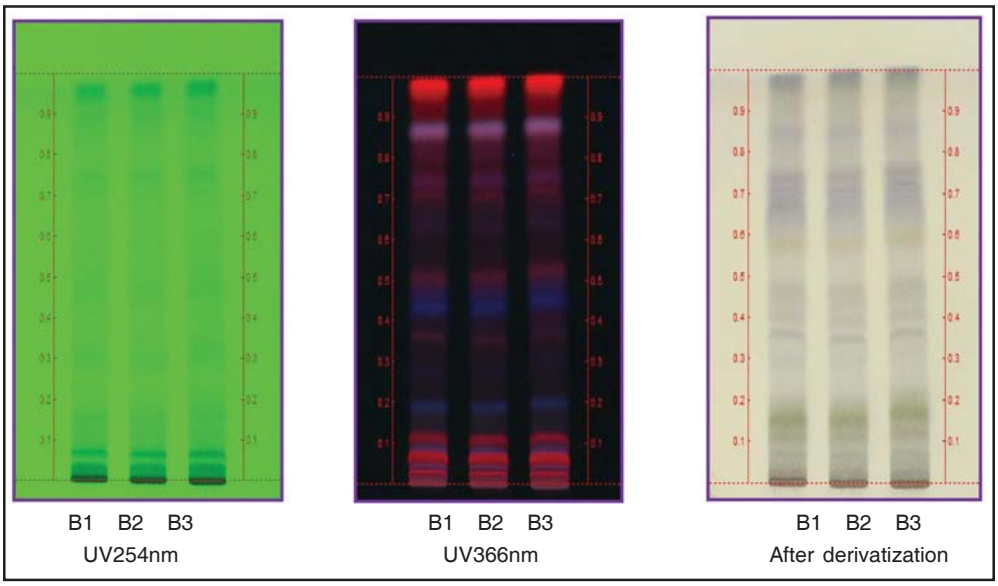


Fig. 7, 8, 9: TLC of Ethanol extract of Habb-e-Banafsha

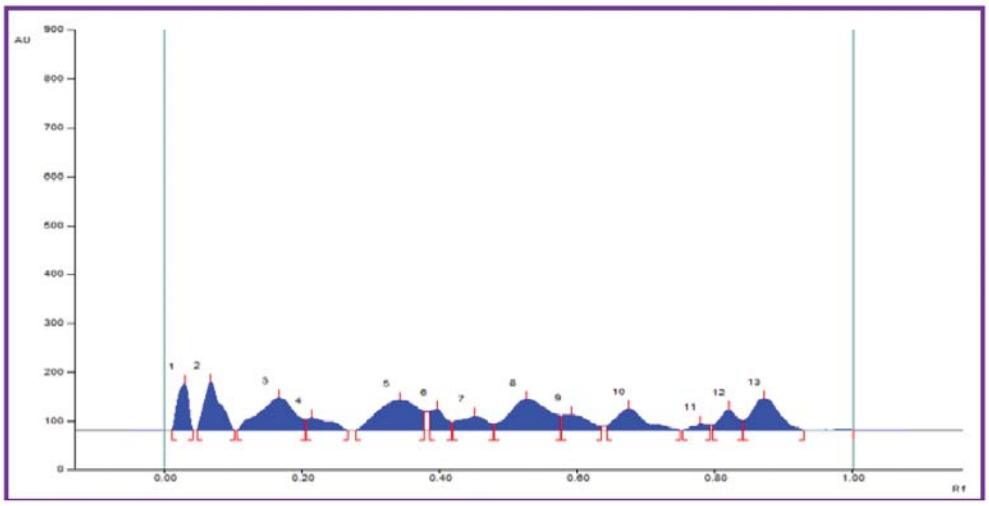


Fig. 10: HPTLC Finger Printing of Ethanol extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.2 AU	0.03 Rf	96.2 AU	14.14 %	0.04 Rf	0.0 AU	1028.1 AU	5.95 %
2	0.05 Rf	1.5 AU	0.07 Rf	98.3 AU	14.44 %	0.10 Rf	0.2 AU	1618.3 AU	9.36 %
3	0.11 Rf	0.5 AU	0.17 Rf	66.2 AU	9.72 %	0.21 Rf	22.5 AU	2408.8 AU	13.93 %
4	0.21 Rf	22.7 AU	0.22 Rf	25.7 AU	3.77 %	0.27 Rf	0.1 AU	614.9 AU	3.56 %
5	0.28 Rf	0.5 AU	0.34 Rf	61.4 AU	9.03 %	0.38 Rf	39.4 AU	2589.9 AU	14.98 %
6	0.39 Rf	38.8 AU	0.40 Rf	42.6 AU	6.26 %	0.42 Rf	16.3 AU	659.3 AU	3.81 %
7	0.42 Rf	16.5 AU	0.45 Rf	28.9 AU	4.24 %	0.48 Rf	13.3 AU	825.0 AU	4.77 %
8	0.48 Rf	13.5 AU	0.53 Rf	63.8 AU	9.37 %	0.58 Rf	30.5 AU	2570.9 AU	14.87 %
9	0.58 Rf	30.6 AU	0.59 Rf	32.5 AU	4.78 %	0.64 Rf	8.4 AU	829.2 AU	4.80 %
10	0.64 Rf	10.0 AU	0.68 Rf	45.0 AU	6.62 %	0.75 Rf	0.0 AU	1325.6 AU	7.67 %
11	0.75 Rf	0.2 AU	0.78 Rf	12.6 AU	1.85 %	0.79 Rf	11.5 AU	199.1 AU	1.15 %
12	0.80 Rf	10.8 AU	0.82 Rf	42.1 AU	6.19 %	0.84 Rf	20.1 AU	762.6 AU	4.41 %
13	0.84 Rf	20.2 AU	0.87 Rf	65.3 AU	9.60 %	0.93 Rf	0.2 AU	1858.5 AU	10.75 %

Fig. 10: R_f Values of HPTLC Finger prints of Ethanol extract

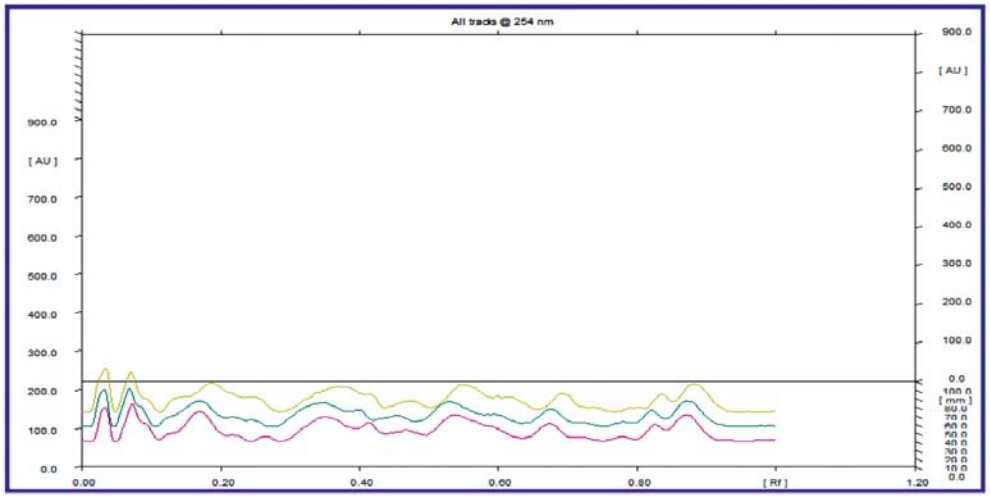
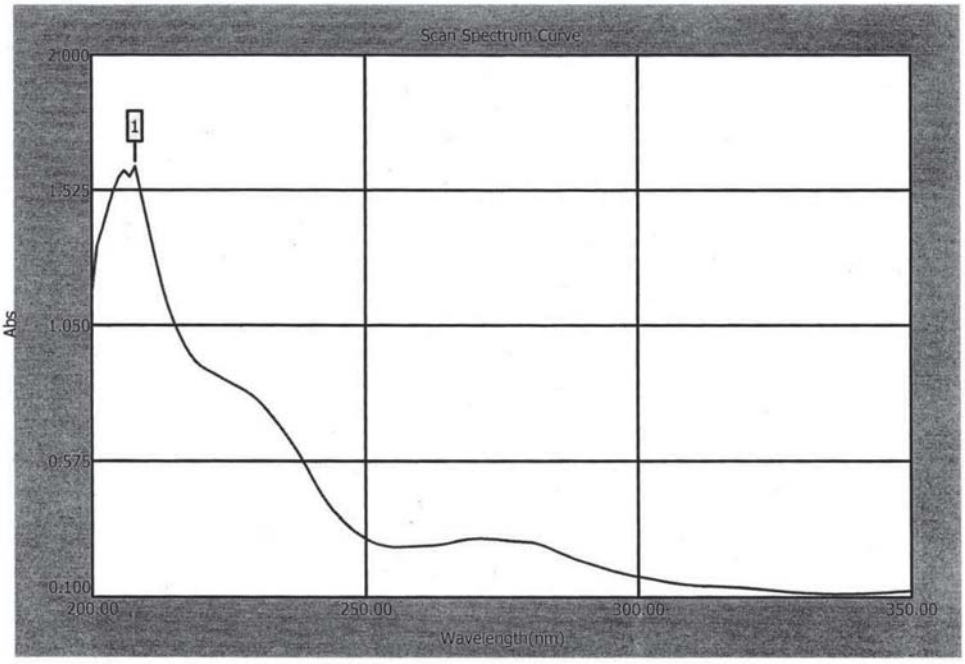


Fig. 11: HPTLC Chromatogram of Ethanol extract

UV Spectroscopic Studies

The study of the U.V. spectra of Habb-e-Banafsha and its ingredients shows that a broad spectrum at 208nm with an absorbance of 1.609% appears in the spectrum of Habb-e-Banafsha (Fig. 12). This single broad spectrum indicates the merger of characteristic peaks of its ingredients as its ingredients show a sharp peak at 206nm with an absorbance of 1.090%(Banafsha), a sharp peak at 206nm with an absorbance of 1.158%(Rubb-us-soos) and a sharp peak at 208nm with an absorbance of 1.540%(Turbud) (Fig.13, 14 & 15).It is clearly evident that each drug has its specific data regarding maximum absorption peak and also has characteristic spectra which can be applied for the prediction of authenticity of the drug.

Habb-e-Banafsha



● **Instrument Performance**
Model : TU-1810DASPC
Number : 18-1885-01-0079
Spectral Bandwidth : 2.00 nm

● **Scan Spectrum Performance**
Scan Range : 200.00 to 350.00 nm
Measure Mode : Abs
Interval : 1.00 nm
Speed : Medium
Data File : Habb e Banafsha 1g in 100ml pet ether.spd
Create Date/Time : Tuesday, March 31, 2015 11:47:16 AM
Data Type : Original
Method File:

● No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	208.00	1.609	

Fig. 12: UV Spectrum of Habb-e-Banafsha

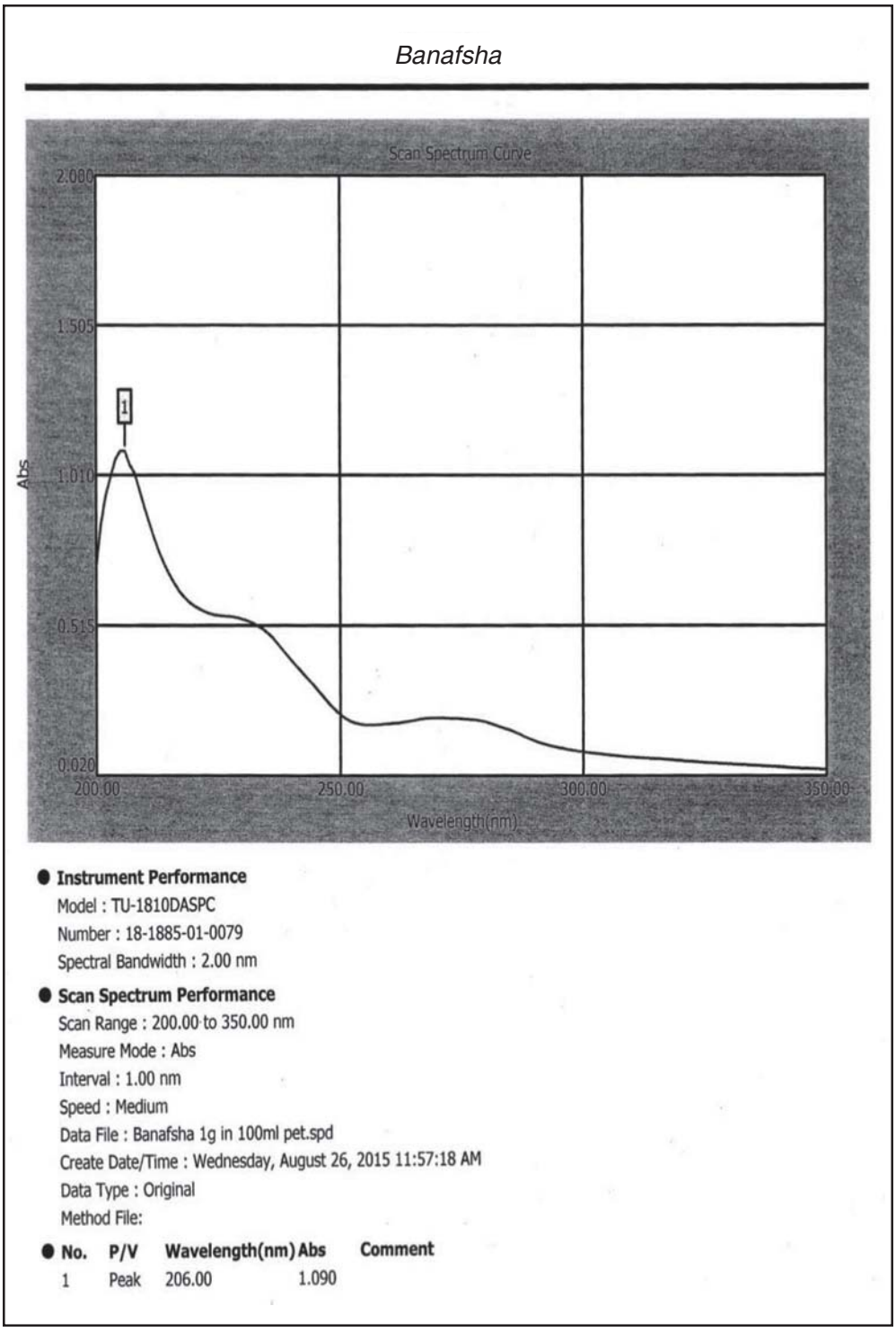


Fig. 13: UV Spectrum of Banafsha

Conclusion

It can be concluded that organoleptic parameters are not much reliable in identification of herbal drugs once the ingredients are powdered and mixed together for preparing compound formulation. The present study, therefore, holds

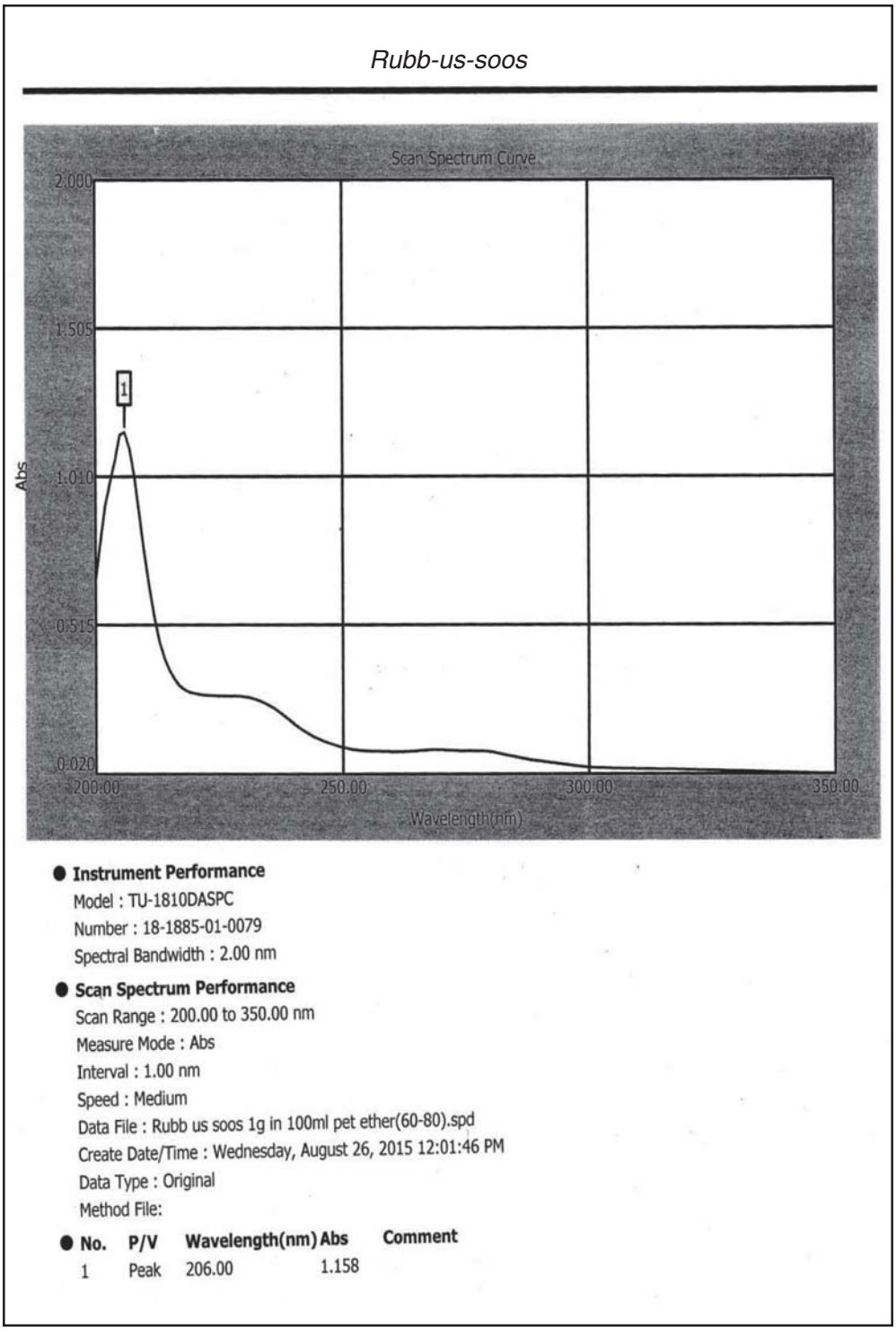


Fig. 14: UV Spectrum of Rubb-us-Soos

high significance as the microscopic features, various Physico-chemical parameters, HPTLC profile, UV spectrum etc. provide criteria for easy identification of the drug Habb-e-Banafsha and ensure the authenticity, quality and efficacy of the medicine.

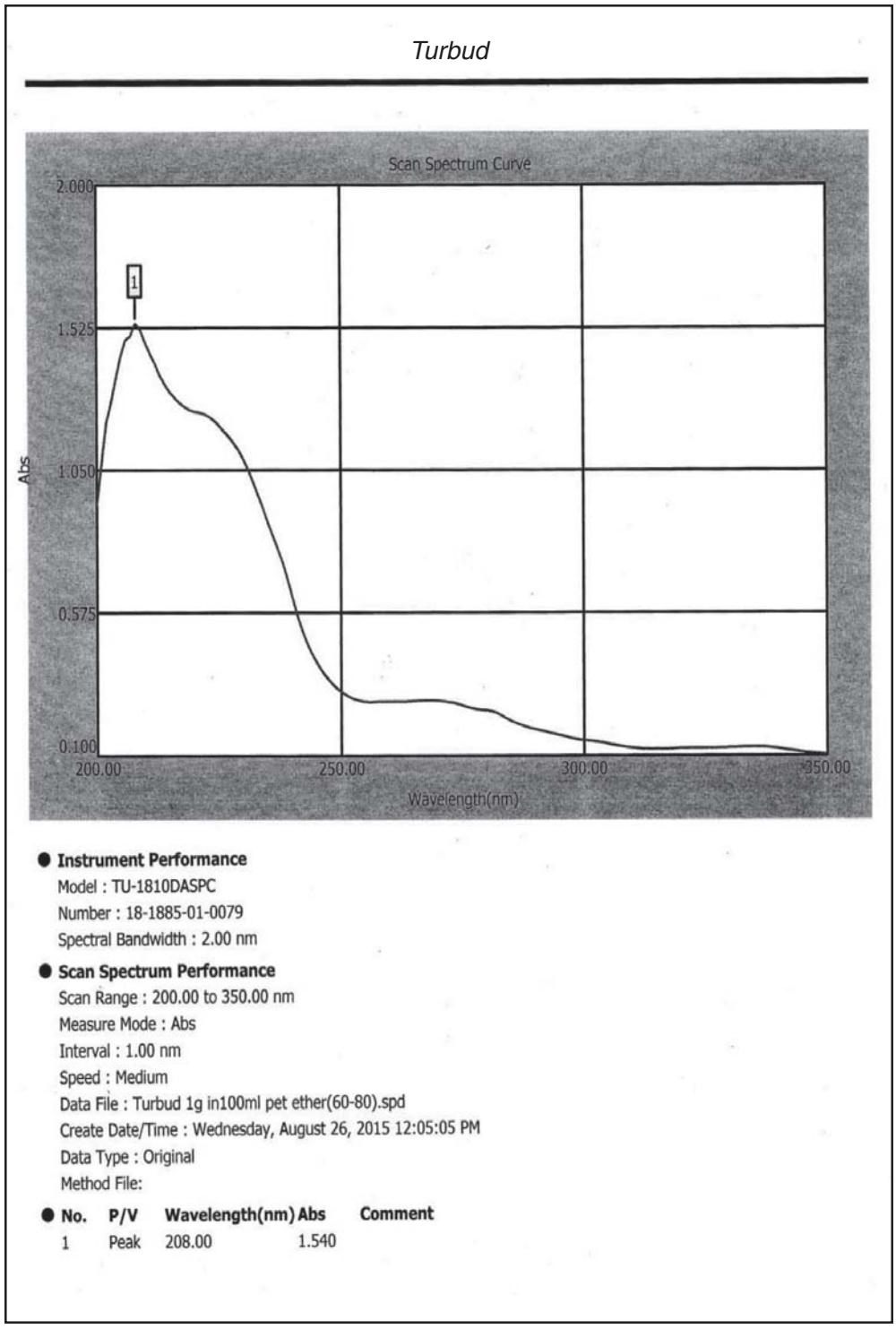


Fig. 15: UV Spectrum of Turbud

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