Standardization of Polyherbal Unani Formulation – Habb-e-Bawaseer Badi

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Abstract

Standardization is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. Most of the traditional medicines are effective but still they are lacking in standardization. There is a need to develop a standard technique to standardize the herbal formulation so as to assess the quality, purity, safety and efficacy of the herbal formulation. The drug Habb-e-Bawaseer Badi is therapeutically useful in the treatment of Bawaseer Badi (Non-bleeding piles). The drug Habb-e-Bawaseer Badi was prepared in three different batches as per the guidelines of National Formulary of Unani Medicine (Part-VI). Present study was aimed to evaluate the pharmacopoeial standards using physico-chemical parameters; TLC/HPTLC fingerprints and quality control parameters using WHO guideline to ascertain the quality of drug. The physico-chemical data showed that the drug contains moisture (2.37%), total ash (11.08%), acid in-soluble ash (5.17%) solubility in alcohol (12.99%) and water (28.13%). TLC/HPTLC finger prints showed various spots at 254nm, 366nm and visible light (V-S reagent). The quality control study revealed the absence of microbial load, aflatoxins, heavy metal and pesticide residues. The evaluated standards will be much useful for laying down the pharmacopoeial standards of Habb-e-Bawaseer Badi and also in providing the quality medicine to the needy masses.

Keywords: Habb-e-Bawaseer Badi, Physicochemical, TLC and WHO parameters

Introduction

Standardization of herbal drugs is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season, area of collection, extraction, purification process and rationalizing the combination in the case of polyherbal drugs (Patel *et al.,* 2006).

The subject of standardization of herbal drug is massively wide and deep. There are many seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function (Yadev *et al*., 2011). For the purpose of research work on standardization of herbal formulations, a profound knowledge is of utmost important.

Historically, herbal medicines have played a significant role in the management of both minor and major medical illness (Bahuguna *et al*., 2014). The quality

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control of herbal crude drugs and formulations is important in justifying their acceptability in modern system of medicine. Hence there is a need to conduct the research on standardisation to provide the effective and safe drugs to the needy mass suffering from various the ailments.

The drug Habb-e-Bawaseer Badi (Anonymous, 2006) is one of the classical Unani poly-herbal compound formulations. It is therapeutically useful in the ailments of Bawaseer Badi (Non-bleeding piles). The preparation of the drug in different batches is based on traditional methods in accordance with the procedure given in NFUM, Part-VI (Anonymous, 2011).

The present paper deals with preparation and standardization of the herbal formulation using various pharmacopoeial parameters such as organoleptic characters, microscopical, physico-chemical, TLC and quality control parameters.

Material and Methods:

Ingredients used for preparation

The raw drugs namely Samagh-e-Dhak, Jadwar, Pakhanbed, Dana Heel Kalan, Filfil Siyah, Zeera Safaid, Gul-e-Surkh, Tabasheer, Rewand Chini, Tukhm-e-Gandana, Post-e-Halela Zard, Post-e-Balela, Aamla Khushk, Sandal Surkh, Sandal Safaid, Kat Safaid, Berg-e-Neem, Berg-e-Hina, Rasaut Zard and Arq-e-Mundi of the formulation were procured from different raw drug dealers from Chennai and Hyderabad. These raw drugs were authenticated as per pharmacopoeial and other official standards (Anonymous, 1990, 2007, 2008 & 2009).

Drug preparation

The drug was prepared in different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM-VI (Anonymous, 2011). The required quantities of all the ingredients were taken of pharmacopoeial quality. The raw drugs 1 to 19 (Table-1) were cleaned, dried, powdered and sieved through 100 meshes and kept separately. Then all the powdered ingredients (1 to 19) in required proportions were mixed thoroughly and kept separately. The ingredient number 20 was added to the mixed powders and again mixedthoroughly to prepare the lubdi mass. Lubdi mass was rolled and converted into sticks of required size and thickness and were cut into small pieces using a knife. Then the cut pieces were rounded between fingers to give the shape of Huboob of required size and weight. The Huboob were dried under shade and stored in a tightly closed glass container.

Table 1: List of Ingredients used in Habb-e-Bawaseer Badi

S.No.	Unani Name	Botanical Name	Part Used	Quantity
1.	Samagh-e-Dhak	*Butea monosperma* (Lam.) Kuntze	Gum	30g
2.	Jadwar	*Delphinium denudatum* Wall.	Root	30g
3.	Pakhanbed	*Bergenia ciliate* (Haw.) Stemb	Rhizome	30g
4.	Dana-e-Heel Kalan	*Amomum subulatum* Roxb.	Seed	30g
5.	Filfil Siyah	*Piper nigrum* Linn	Fruit	30g
6.	Zeera Safaid	*Cuminum cyminum* Linn.	Fruit	30g
7.	Gul-e-Surkh	*Rosa damascena* Mill.	Flower	30g
8.	Tabasheer	*Bambusa bambos* Druce.	Bamboo Manna	30g
9.	Rewand Chini	*Rheum emodi* Wall.	Root	30g
10.	Tukhm-e-Gandana	*Asphodelus tenuifolius* Cav.	Seed	60g
11.	Post-e-Halela Zard	*Terminalia chebula* Retz.	Pericarp of the fruit	60g
12.	Post Balela	*Terminalia bellerica* Roxb.	Pericarp of the fruit	60g
13.	Aamla Khushk	*Emblica officinalis* Gaertn.	Pericarp of the fruit	60g
14.	Sandal Surkh	*Pterocarpus santalinus* Linn	Heart wood	60g
15.	Sandal Safaid	*Santalum album* Linn.	Heart wood	60g
16.	Kat Safaid	*Acacia leucophloea* Willd.	Stem bark	60g
17.	Barg-e-Neem	*Azadirachta indica* A. Juss.	Leaves	60g
18.	Barg-e-Hina	*Lawsonia inermis* Linn.	Leaves	60g
19.	Rasaut Zard	*Berberis aristata* DC.	Root extract	75g
20.	Araq-e-Mundi	*Sphaeranthus indicus* Linn.	Distilate	Q. S.

Pharmacopoeial standards

Pharmacopoeial studies such as organoleptic characters, microscopical, physico-chemical, TLC/HPTLC and quality control parameters were carried out

1. Organoleptic Evaluation: Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc., using the sensory organs of our body. The organoleptic characters of the samples were carried out based on the method described by Siddique *et al.* (1995).

2. Physico-chemical analysis: If the water content is high the drug can easily be deteriorated due to fungus. The ash content indicates the total amount of inorganic material after complete incineration and the acid insoluble ash is an indicative of silicate impurities might be due to improper washing of the drug. The alcohol and water soluble extractive indicates the amount of active chemical constituents in a given amount of particular drug when extracted with respective solvent The moisture content of the powdered sample at 105°C, ash values, acid insoluble ash, solubility in water and alcohol, pH values and bulk density etc., are the useful tools in standardisation of herbal products were studied as per standards method (Anonymous, 1987).

3. TLC/HPTLC finger print analysis: The drug samples (2g) were soaked in chloroform and alcohol separately for 18 hours and refluxed for ten minutes on water bath and filtered through Whatmann No. 1 filter paper. The filtrates were concentrated and made up to 10ml in volumetric flask with respective solvents **(**Saxena and Yadav, 1983). The procedure followed for the analysis of TLC and HPTLC was as per the standard method (Wagner and Bladt, 1996).

4. Quality control parameter: The usage of herbal products along with higher safety margins, WHO has taken necessary step to ensure quality control parameters with the modern techniques and application of suitable standards. The microbial load and heavy metal parameters using the powder were carried out as per the WHO guidelines (Anonymous, 1998). The heavy metals were analysed by Atomic Absorption Spectroscopy (Anonymous, 2005); aflatoxins were estimated by Kobra cell technique using Agilent HPLC instrument as per the method ASTA (Anonymous, 1997) and pesticide residues were analysed using GC-MS Agilent instrument equipped with Mass selective detector as per the method AOAC (Anonymous, 2005).

Results and Discussion

Organoleptic character of the drug indicates that the drug is dark brown colour, cumin odour and bitter taste. The physico-chemical analysis such as LOD obtained in the drug was 2.37% shows the amount of moisture content present in the drug. The alcohol soluble extractive (12.99%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (28.13%) indicate the presence of inorganic constituents (Table – 2).

Thin Layer Chromatography

TLC/HPTLC profile of chloroform extract – TLC/HPTLC profile of chloroform extract was developed using Toluene: Ethyl acetate: Formic acid (8.5: 1.5: 0.2) as mobile

Table 2: Physico-chemical parameters

Parameters Analyzed	Batch Number		
	I	II	III
Extractives Alcohol soluble matter Water soluble matter	 13.00% 28.19%	 12.97% 28.10%	 12.99% 28.11%
Ash values Total ash Acid insoluble ash	 11.11% 5.24%	 11.03% 5.10%	 11.09% 5.17%
pH values 1% Aqueous solution 10% Aqueous solution	 4.75 4.64	 4.75 4.64	 4.75 4.64
Moisture content	2.36%	2.38%	2.37%
Alcohol soluble extractive values (Soxhlet – Hot extraction)	14.97%	14.64%	14.73%
Chloroform soluble extractive values (Soxhlet – Hot extraction)	13.36%	13.23%	13.29%
Bulk density gm/ml	0.6424	0.6419	0.6441

phase. TLC profile at UV-254 nm, UV-366 nm and after derivatized with vanillin - sulphuric acid shown in Fig. - 1 & Table – 3 with 9 spots, 22 spots and 14 spots respectively. HPTLC finger print profile of chloroform extract was scanned at 254nm showed 13 peaks (Fig.- 2) of which 7 were major peaks at R_f value of 0.18, 0.23, 0.26, 0.30, 0.56, 0.88 and 0.98 whereas others were moderately smaller peaks.

The Densitometric chromatograms of chloroform extract for different batch samples of Habb-e-Bawaseer Badi were taken and shown in (Fig. 3).

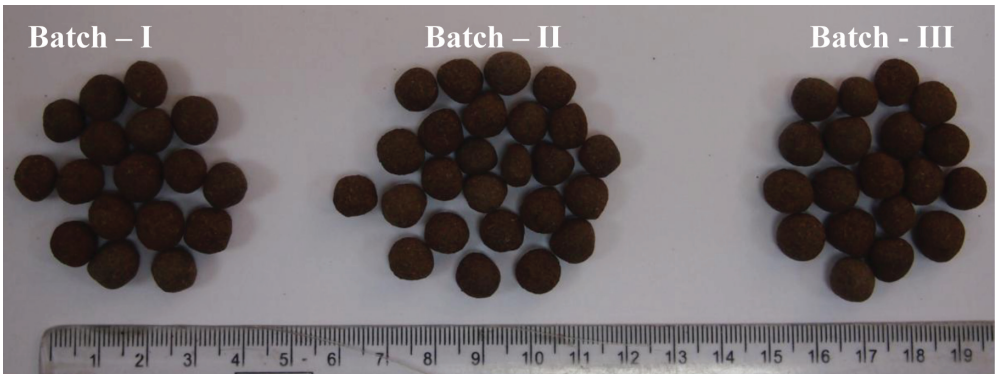
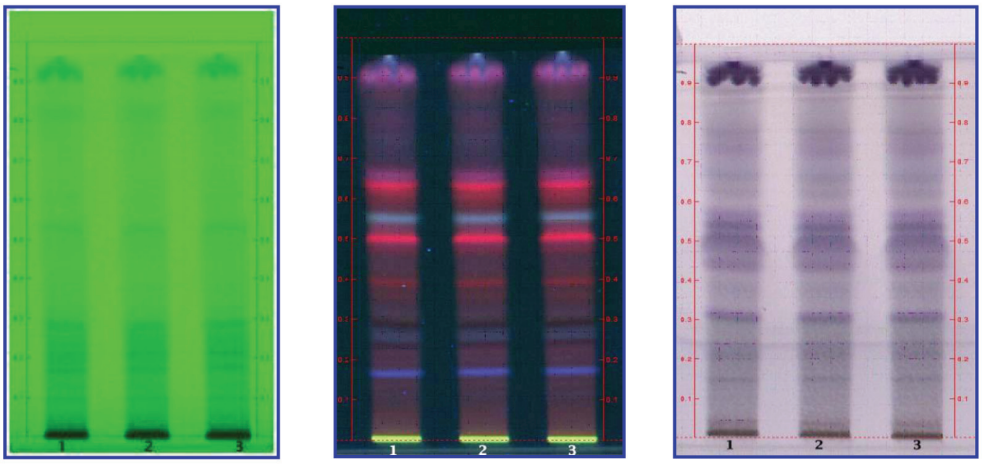


Figure 1: Habb-e-Bawaseer Badi

Table 3: R_f values of chloroform extract

Solvent system	R_f Values		
	254nm	366nm	VS reagent
Toluene : Ethyl acetate : Formic acid (8.5 : 1.5 : 0.2)	0.82 Green	0.91 Pink	0.88 Grey
	0.69 Green	0.88 Red	0.79 Grey
	0.53 Green	0.85 Violet	0.75 Violet
	0.28 Green	0.79 Red	0.71 Grey
	0.25 Green	0.76 Violet	0.68 Blue
	0.22 Green	0.69 Red	0.61 Grey
	0.18 Green	0.66 Pink	0.59 Violet
	0.15 Green	0.64 Red	0.54 Dark blue
	0.10 Green	0.61 Brown	0.50 Dark blue
		0.58 Blue	0.48 Dark blue
		0.52 Pink	0.35 Grey
		0.50 Red	0.31 Violet
		0.48 Red	0.23 Grey
		0.45 Violet	0.15 Grey
		0.41 Brown	
		0.36 Brown	
		0.32 Violet	
		0.30 Dark brown	
		0.28 Grey	
		0.26 Brown	
		0.19 Violet	
		0.10 Light grey	



UV - 254nm UV - 366nm V - S Reagent

Solvent System: Toluene : Ethyl acetate : Formic acid (8.5 : 1.5 : 0.2)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III

Figure 2: TLC/HPTLC Photo of Chloroform Extract

Table 4: R_f values of alcohol extract

Solvent system	R_f Values		
	254nm	366nm	VS reagent
Toluene : Ethyl acetate (9 : 1)	0.91 Green	0.94 Blue	0.89 Dark grey
	0.79 Green	0.75 Brown	0.77 Dark blue
	0.72 Green	0.65 Brown	0.72 Grey
	0.61 Green	0.60 Blue	0.67 Grey
	0.51 Green	0.57 Red	0.61 Grey
	0.49 Green	0.54 Red	0.51 Grey
	0.40 Dark green	0.49 Red	0.48 Grey
	0.31 Dark green	0.47 Blue	0.41 Dark blue
	0.28 Dark green	0.44 Pink	0.35 Grey
	0.19 Dark green	0.41 Violet	0.33 Grey
	0.15 Dark green	0.39 Red	0.25 Violet
	0.09 Dark green	0.35 Brown	0.24 Dark blue
		0.31 Red	0.21 Grey
		0.29 Pink	0.15 Dark green
		0.27 Blue	
		0.23 Grey	
		0.21 Yellow	
		0.19 Blue	
		0.17 Grey	
		0.15 Blue	
		0.13 Pink	
		0.10 Brown	

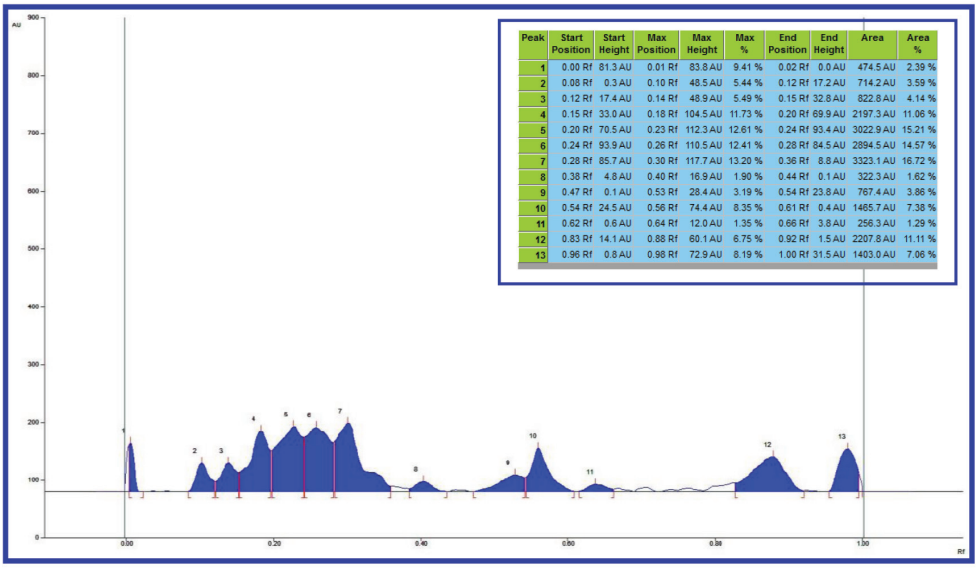


Figure 3: HPTLC Fingerprint of chloroform extract at 254nm

TLC/HPTLC profile of alcohol extract was developed using Toluene: Ethyl acetate (9: 1) as mobile phase. TLC profile at UV-254 nm, UV-366 nm and after derivatized with vanillin - sulphuric acid has shown in (Fig. 4) & Table-4 with 12 spots, 22 spots and 14 spots respectively.

HPTLC finger print profile of alcohol extract was scanned at 254nm showed 12 peaks (Fig. 5) of which 5 were major peaks at R_f value of 0.10, 0.16, 0.27, 0.33 and 0.42 whereas others were moderately smaller peaks.

Densitometric chromatograms of alcohol extract were taken for different batch samples of Habb-e-Bawaseer Badi (Fig.- 6).

Quality Control Parameters

The analysis of microbial load present in the drug showed that the total bacterial count (TBC) and total fungal count (TFC) was 700 and 200 CFU/gm. The detection of the microbial load was under the permissible limits of WHO guideline

Table 5: Analysis of Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	700 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	200 CFU / gm	10^3 CFU / gm
3	Enterobacteriaceae	Absent	10^3 CFU / gm
4	*Salmonella* spp.	Absent	Absent
5	*Staphylococcus aureus*	Absent	Absent

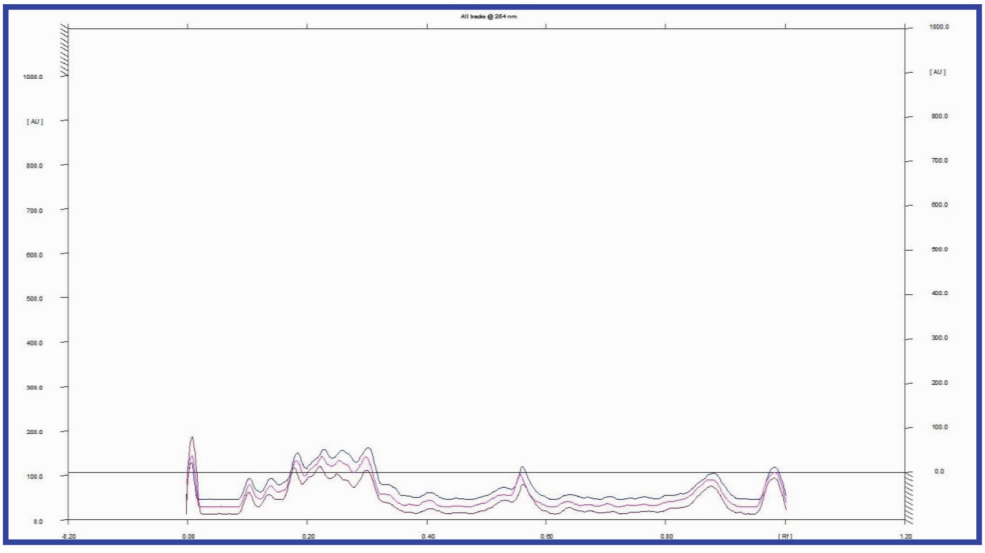
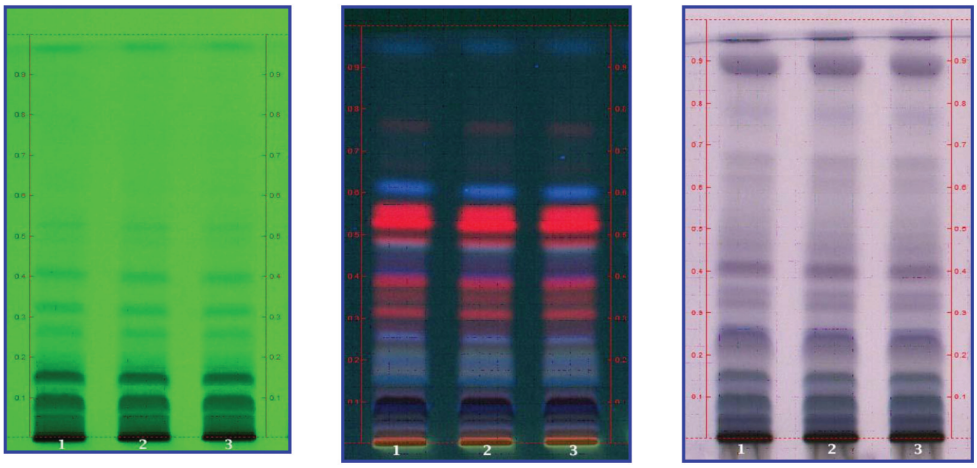


Figure 4: Densitometric chromatogram of chloroform extracts at 254nm

Table 6: Estimation of Heavy Metals

S.No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Lead	5.10ppm	10 ppm
2	Cadmium	Not detected	0.3 ppm
3	Mercury	Not detected	1 ppm
4	Arsenic	Not detected	3 ppm



UV – 254nm UV - 366nm V - S Reagent

Solvent System: Toluene : Ethyl acetate (9 : 1)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III

Figure 5: TLC/HPTLC photo of alcohol extract

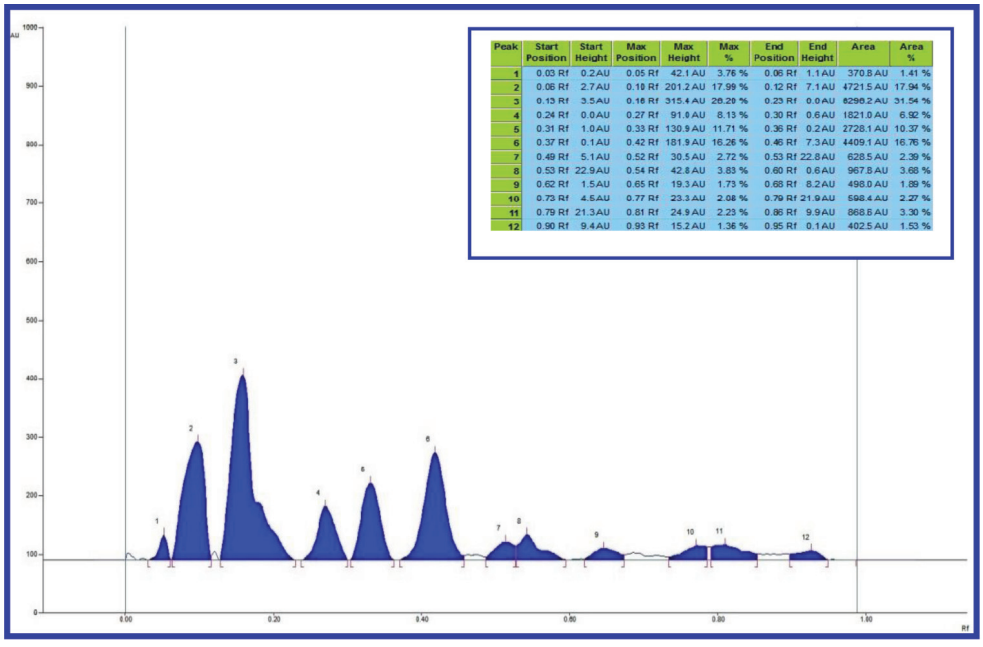


Figure 6 : HPTLC finger print of alcohol extract at 254nm

Table 7: Estimation of Aflatoxins

S.No.	Aflatoxins	Results
1	B1	Not Detected
2	B2	Not Detected
3	G1	Not Detected
4	G2	Not Detected

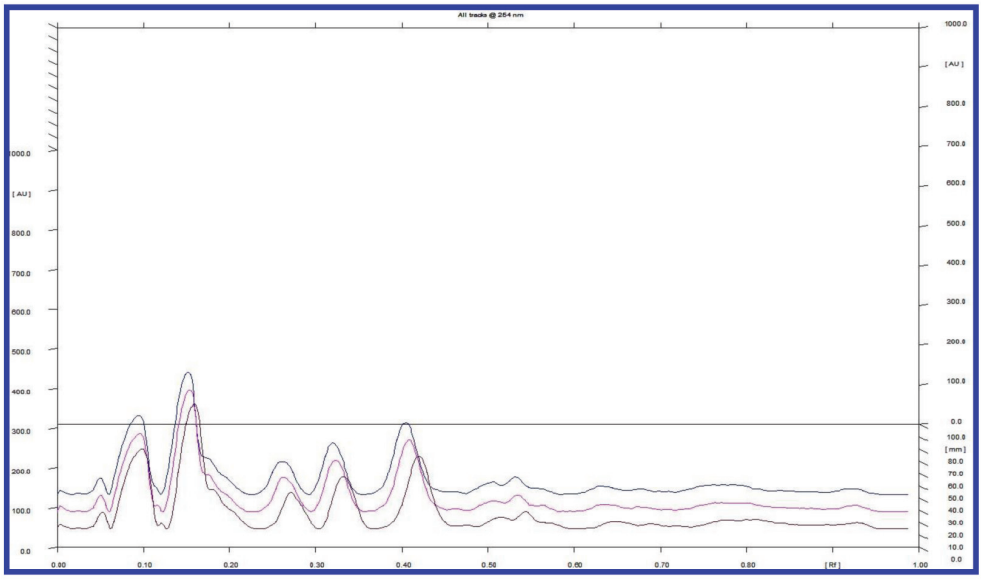


Figure 7 : Densitometric chromatogram of alcohol extracts at 254nm

(Anonymous, 1998). At the same time the bacteria *Enterobacteriaceae*, *Salmonella* spp, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* spp were found to be absent in the drug which indicated that the drug is not the carrier of these microorganisms.

The heavy metals likes lead, cadmium, mercury and arsenic which are toxic metals can either be unintentional or intentional, in herbal preparation were analyzed using Atomic Absorption Spectrophotometer (AAS) showed the presence of lead 5.10ppm within the permissible limit (Anonymous, 2008) and other metals were found absent from the drug. The analysis of aflatoxins in the drug using HPLC showed that the aflatoxins B_1, B_2, G_1 and G_2 found below the detecting limit and hence the present study shows the non-toxic effect of the drug.

The analysis of various pesticidal residues like HCH (all isomers), DDT (all isomers), DDE (all isomers), Endosulphan (all isomers) etc., were tested in the drug. All the above mentioned pesticides were not detected.

Table 8: Analysis of Pesticide Residues

S.No.	Pesticide Residues	Results
1	Organo Chlorine group	Not Detected
2	Organo Phosphorus group	Not Detected
3	Acephate	Not Detected
4	Chlordane	Not Detected
5	Dimethoate	Not Detected
6	Endosulphan	Not Detected
7	Endosulfan	Not Detected
8	Endosulfon	Not Detected
9	Ethion	Not Detected
10	Endosufon sulphate	Not Detected
11	Fenthion	Not Detected
12	Heptachlor	Not Detected
13	Lindane	Not Detected
14	Methoxychlor	Not Detected
15	Phorate sulfoxide	Not Detected
16	Phorate sulfone	Not Detected

Conclusion

In the present investigation various standardization parameters such as physico-chemical, TLC/HPTLC finger prints and WHO parameters were carried out can be laid down as reference standards of the drug Habb-e-Bawaseer. From the present study it can be concluded that the formulation Habb-e-Bawaseer Badi is safe, effective and economic drug and the efficacy of the drug can be evaluated by conducting the clinical studies on patients surfing piles dease.

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