Development of Quality Standards on Jawarish-e-Kafoor Qawi – A Classical Unani Formulation#

[1]*Rampratap Meena,
[2]S. Mageswari,
[2]D. Ramasamy,
[2]P. Meera Devi Sri,
[3]Shamsul Arfin,
[3]Aminuddin
and
[4]Nitin Rai

[1]Drug Standardisation Research Institute,
PLIM Campus, Sanjay Nagar,
Ghaziabad-201002

[2]Regional Research Institute of Unani Medicine,
1, West Madha Church Street,
Royapuram, Chennai-600013

[3]Central Council for Research in Unani Medicine,
61-65 Institutional Area,
Janakpuri, New Delhi-110058

[4]Pharmacopoeial Laboratory for Indian Medicine,
Kamla Nehru Nagar,
Ghaziabad-201002

Abstract

The Unani system of medicine prescribes large number of classical herbal formulations to cure the different types of diseases. Jawarish-e-Kafoor Qawi a Unani herbal formulation is prepared in combination of ingredients like Kafoor, Zafran, Jauzbuwa, Filfil Siyah, Zanjabeel, Bisbasa, Darchini, Narmushk, Qirfa, Filfilmoya, Faranjmushk and Qand Safaid. The Unani Physicians prescribes the drug Jawarish-e-Kafoor Qawi to cure the ailments of Zof-e-Meda (Weakness of the stomach) and Nafkh-e-Shikam (Flatulence in the stomach). At present no pharmacopoeial standards on drug is available and it is basic requirement for the research on quality control of this drug. There is lack of standardization and proper documentation of Unani drugs. Based on the available sources an attempt is made to evaluate the drug on pharmacopoeial parameters to develop standards for the drug Jawarish-e-Kafoor Qawi. To evaluate the pharmacopoeial parameters of the drug, various parameters like powder microscopy, moisture content, ash values, bulk density, pH values, extractive values, TLC/HPTLC finger printing and other quality control parameters viz. heavy metals, microbial content, aflatoxins and pesticide residues are performed. The evaluated data will help to lay down pharmacopoeial standards for the drug 'Jawarish-e-Kafoor Qawi'.

Keywords: Jawarish-e-Kafoor Qawi, Powder microscopy, Physico-chemical, TLC/HPTLC, WHO parameters

Introduction

Jawarish-e-Kafoor Qawi (Anonymous, 2006) is one of the ancient commonly used classical Unani formulations. This poly herbal formulation consists of 12 ingredients (Table 1). This drug is prescribed for the treatment of Zof-e-Meda (weakness of the stomach) and Nafkh-e-Shikam (flatulence in the stomach) disorders. The development of traditional medicines particularly Unani medicines with the perspective of safety, efficacy and quality will not only to preserve the traditional heritage but also to rationalize the uses of Unani medicines in the health care.

Standardisation of Unani herbal formulations is necessary step to assess the quality of drugs. Due to lack of Standard Operating Procedures (SOP's) and quality control methods, there are batch to batch variations among the similar formulations. Pharmacopoeial study of a drug is an essential requirement to establish the presence of each ingredient in the formulations (Bandaranayake,

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[1]*Author for correspondence

2006; Myers and Cheras, 2004). The present study is an attempt to evaluate the pharmacopoeial studies of the drug by applying modern parameters such as microscopical, physico-chemical, thin layer chromatography and WHO parameters such as microbial load, aflatoxin, heavy metals and pesticide residue.

Material and Methods

To evaluate the pharmacopoeial studies of Jawarish-e-Kafoor Qawi a systematic scheme of standardization was developed.

(i) Ingredients authentications

Genuine raw drugs namely Kafoor, Zafran, Jauzbuwa, Filfil Siyah, Zanjabeel, Bisbasa, Darchini, Narmushk, Qirfa, Filfilmoya, Faranjmushk and Qand Safaid of the formulation were procured from raw drugs dealers of Chennai and Delhi (Fig. 1). The raw drugs were authenticated as per pharmacopoeial and other official standards (Anonymous, 2004, 2007, 2008 & 2009).

(ii) Drug formulation

The ployherbal semisolid drug was prepared in different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM, Part – IV (Anonymous, 2006) (Table 1).

(iii) Powder microscopy

The drug sample (5g) was weighed and mixed with 50ml of water in a beaker with gentle warming, till the sample completely dispersed in water. The mixture was centrifuged and decanted the supernatant. The sediment was washed several times with distilled water, centrifuged again and decanted the supernatant. A few mg of the sediment was taken and mounted in glycerine. A few mg was taken in watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid, mounted in glycerine. The microscopic salient features of the drug were observed in different mounts (Wallis, 1997; Johansen, 1940).

(iv) Physico-chemical analysis

The physico-chemical methods viz., moisture content, ash values, solubility in different solvents, pH values, bulk density and sugar content etc., are useful tools in standardisation of a herbal product for maintaining batch to batch consistency. The drug samples were subjected for the standardisation of physico-chemical parameters and analysed as per the standards method (Anonymous, 1987).

Fig. 1: Ingredients of Jawarish-e-Kafoor Qawi

(v) TLC/HPTLC finger printing

The formulations of the three batch samples were extracted with chloroform and alcohol. The extracts were concentrated and made up to 10 ml in a

volumetric flask separately. These solutions were used for the TLC/HPTLC finger print analysis.

The TLC/HPTLC finger print analysis of chloroform and alcohol extracts of the formulations were performed using aluminium plate precoated with silica gel 60 F_{254} (E.merck) employing CAMAG Automatic TLC sample - IV applicator. The chromatogram were developed using the developing systems toluene: ethyl acetate (9: 1) and toluene: ethyl acetate (6: 4) for chloroform and alcohol extracts respectively. The plates were dried at room temperature to record the image of the plates at UV-254 nm, UV-366 nm using TLC visualizer and the plates were scanned at 254 nm to record the finger print spectrum using TLC Scanner - IV. Finally the plate were dipped in vanillin-sulphuric acid and heated at 105° till coloured spots appeared (Wagner, and Bladt, 1984; Sethi, 1996).

(vi) Other 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 were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxin and pesticide residues were carried out by standard methods (Anonymous, 2000).

Obseravtions

Jawarish-e-Kafoor Qawi is a dark brown semi-solid product with sweetish bitter taste.

(i) Pharmacognostical observation (Powder microscopy):

The diagnostic characteristics of cellular elements in respect of each ingredients is in Table 1 and Fig. 2.

(ii) Chemical analysis

The physico-chemical data such as moisture content was obtained in the drug 19.57%. The alcohol soluble extractive (44.56%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (65.54%) indicate the presence of inorganic constituents. The obtained data are shown in Table 2.

(iii) Thin Layer Chromatography analysis

The chloroform and alcohol extract of all the three batch samples showed identical spots in UV – 254nm and 366nm ranges and the R_f values of both

Table 1: List of the raw drugs and cellular elements of Jawarish-e-Kafoor Qawi Formulation

S. No.	Unani name	Unani Name	Part used	Quantity	Salient features of the drug
1.	Kafoor API-VI	*Cinnamomum camphora* (L.) Nees & Eberm.	Natural camphor	25 g.	–
2.	Zafran UPI-VI	*Crocus sativus* Linn.	Stamens & Stigmas	25 g.	Pollen grains size upto 120µ, spherical in outline with clear exine and intine
3.	Jauzbuwa UPI-I	*Myristica fragrans* Houtt.	Endosperm	25 g.	Endosperm cells filled with numerous starch grains, crystalline fat and large aleurone grains (crystalloid proteins upto 40µ), perisperm cells filled with reddish brown contents
4.	Filfil Siyah UPI-IV	*Piper nigrum* L.	Fruit	25 g.	Stone cells polygonal interspersed among parenchyma cells with circular lumen, perisperm cells isolated or in groups with angular walls filled with aleurone grains and minute calcium oxalate crystals
5.	Zanjabeel UPI-I	*Zingiber officinale* Rosc	Rhizome	25 g.	Isolated starch grains, simple oval to round shaped measuring upto 70µ, hilum eccentric, lamellae distinct; non-lignified septate fibres upto 50µ, reticulate vessels and fragments of reticulate vessels upto 70µ; parenchyma cells filled with abundant starch grains
6.	Bisbasa UPI-VI	*Myristica fragrans* Houtt.	Arillus	25 g.	Thick walled elongated parenchyma cells in surface view upto 50µ wide
7.	Darchini UPI-I	*Cinnamomum zeylanicum* Blume.	Inner stem bark	25 g.	Fibres thick walled lignified with striated walls and narrow lumen of length upto 1000µ and breadth upto 30µ, stone cells with horse shoe shaped thickenings upto 70µ
8.	Narmushk UPI-IV	*Mesua ferrea* Linn	Stamens	25 g.	Tricolporate golden yellow pollen grains upto 50µ
9.	Qirfa UPI-III	*Cinnamomum cassia* Blume.	Stem bark	25 g.	Fibres thick walled lignified with striated walls and narrow lumen of length upto 1000µ and breadth upto 40µ and very large stone cells upto 200µ, stone cells with horse shoe shaped thickenings upto 70µ
10.	Filfilmoya API-IV	*Piper longum* L.	Fruit	25 g.	Parenchyma cells with elongated spindle shaped stone cells; perisperm cells isolated or in groups with angular walls filled with aleurone grains and minute calcium oxalate crystals
11.	Faranjmushk API-IV	*Ocimum sanctum* L.	Seed	25 g.	Fragments of irregular shaped thick walled epidermal cells
12.	Qand Safaid	Sugar	–	800 g.	–

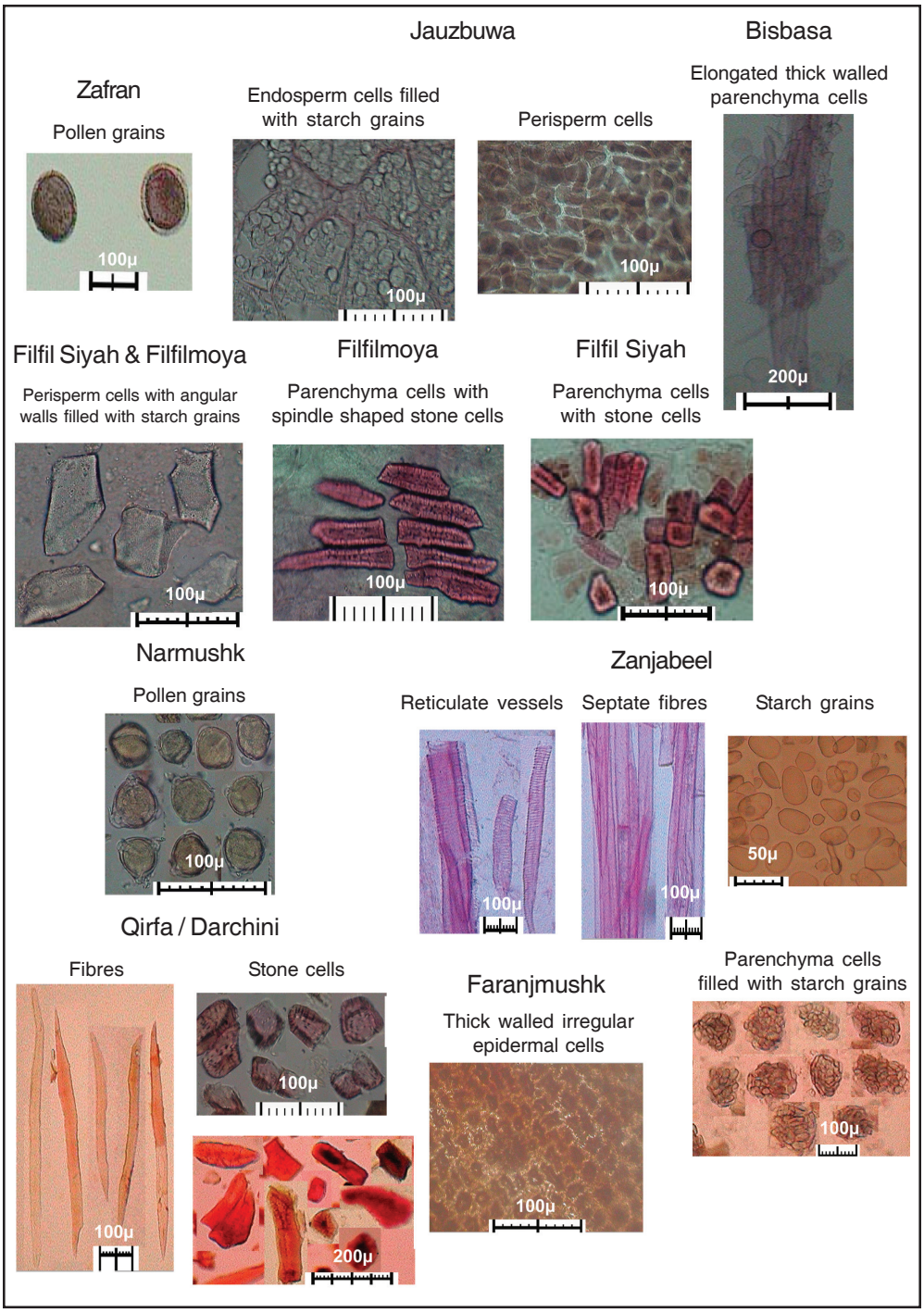

Fig. 2: Powder microscopy of Jawarish-e-Kafoor Qawi

the extracts are shown in Table 3 and 4. The plates were dipped in vanillin-sulphuric acid and heated at 105ºC till appeared coloured spots.

(iv) Quality control parameters

The evaluated quality control parameters such as microbial load and heavy metals were found within the permissible limit in the drug shown in Table 5

Table 2: Physico-chemical parameters

Parameters	Batch Number (n=3)		
	I	II	III
Extractives			
Alcohol soluble matter	44.71%	44.17%	44.80%
Water soluble matter	65.31%	65.79%	65.52%
Ash			
Total ash	0.89%	0.73%	0.62%
Acid insoluble ash	0.18%	0.28%	0.35%
pH values			
1% Aqueous solution	5.36	5.48	5.39
10% Aqueous solution	4.19	4.35	4.41
Sugar estimation			
Reducing sugar	33.61%	33.26%	33.72%
Non-reducing sugar	9.47%	9.09%	9.50%
Moisture	19.24%	19.83%	19.46%
Bulk Density	1.3203	1.3114	1.3152

Table-3: R_f values of the chloroform extract

Solvent System		Rf Values	
	UV-254 nm	UV-366 nm	After derivatisation with vanillin – sulphuric acid reagent
Toluene: Ethyl acetate (9:1)	0.95 Green	0.92 Fluorescent blue	0.93 Grey
	0.80 Green	0.81 Blue	0.83 Violet
	0.72 Green	0.78 Red	0.73 Violet
	0.67 Green	0.69 Red	0.66 Grey
	0.60 Green	0.64 Fluorescent blue	0.59 Grey
	0.48 Green	0.60 Red	0.57 Grey
	0.35 Green	0.56 Red	0.48 Pink
	0.27 Green	0.50 Blue	0.37 Grey
	0.21 Green	0.44 Red	0.32 Violet
	0.16 Green	0.23 Green	0.27 Pink
		0.14 Blue	0.15 Grey

Table 4: R_f values of the alcohol extract

Solvent System		Rf Values	
	UV-254 nm	UV-366 nm	After derivatisation with vanillin – sulphuric acid reagent
Toluene: Ethyl acetate (6:4)	0.92 Green	0.92 Red	0.92 Violet
	0.88 Green	0.86 Pink	0.87 Brown
	0.79 Green	0.78 Blue	0.78 Grey
	0.72 Green	0.59 Green	0.69 Light grey
	0.48 Green	0.51 Green	0.65 Violet
	0.39 Green	0.47 Blue	0.58 Light grey
		0.23 Light blue	0.49 Light grey
		0.10 Pink	0.10 Grey

and 6. The other parameters like aflatoxins B_1, B_2, G_1 and G_2 and pesticide residues - organo chlorine group, organo phosphorus group, acephate, chlordane, dimethoate, endosulphan, endosulfan, endosulfon, ethion, endosufon sulphate, fenthion, heptachlor, lindane, methoxychlor, phorate sulfoxide and phorate sulfone were not detected from the drug samples shown in Table 7 and 8.

Table 5: Analysis of Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	300 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	< 10 CFU/ gm	10^3 CFU / gm
3	Enterobacteriaceae	Absent / gm	10^3 CFU / gm
4	Salmonella	Absent / gm	Nil
5	*Staphylococcus aureus*	Absent / gm	Nil

Table 6: Estimation of Heavy Metals

S.No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Arsenic	Not detected	10 ppm
2	Cadmium	Not detected	0.3 ppm
3	Lead	0.0018 ppm	10 ppm
4	Mercury	Not detected	1.0 ppm

Table 7: Estimation of Aflatoxins

S.No.	Aflatoxins	Results	WHO Limits
1	B1	ND	0.05ppb
2	B2	ND	0.05ppb
3	G1	ND	0.05ppb
4	G2	ND	0.05ppb

ND = Not Detected

Table 8: Analysis of Pesticide Residues

S.No.	Pesticide Residues	Results	Limits
1	Organo Chlorine group	ND	(DL 0.005mg/Kg)
2	Organo Phosphorus group	ND	(DL 0.005mg/Kg)
3	Acephate	ND	(DL 0.005mg/Kg)
4	Chlordane	ND	(DL 0.005mg/Kg)
5	Dimethoate	ND	(DL 0.005mg/Kg)
6	Endosulphan	ND	(DL 0.005mg/Kg)
7	Endosulfan	ND	(DL 0.005mg/Kg)
8	Endosulfon	ND	(DL 0.005mg/Kg)
9	Ethion	ND	(DL 0.005mg/Kg)
10	Endosufon sulphate	ND	(DL 0.005mg/Kg)
11	Fenthion	ND	(DL 0.005mg/Kg)
12	Heptachlor	ND	(DL 0.005mg/Kg)
13	Lindane	ND	(DL 0.005mg/Kg)
14	Methoxychlor	ND	(DL 0.005mg/Kg)
15	Phorate sulfoxide	ND	(DL 0.005mg/Kg)
16	Phorate sulfone	ND	(DL 0.005mg/Kg)
ND – Not detected			

TLC/ HPTLC finger print studies

(i) TLC/ HPTLC finger print studies of chloroform extract

The TLC studies of chloroform extract are tabulated in Table 3. All the three batch samples shows identical spots in UV-254 nm, UV-366 nm and visible light (after derivatised with vanillin – sulphuric acid reagent). In UV – 254, 366 nm and visible light it shows 10, 11 and 11 spots respectively with different R_f values (Fig. 3). The finger print of the chloroform extract shows 13 peaks of

which peaks at R_f 0.17, 0.30, 0.38, 0.57, 0.62, 0.75, 0.82 and 0.91 were the major peak whereas peaks at R_f 0.04, 0.07, 0.22, 0.44 and 0.67 were moderately smaller peaks (Fig.4). The HPTLC densitometry chromatogram of chloroform extract of three batch samples were recorded at 254 nm (Fig. 5).

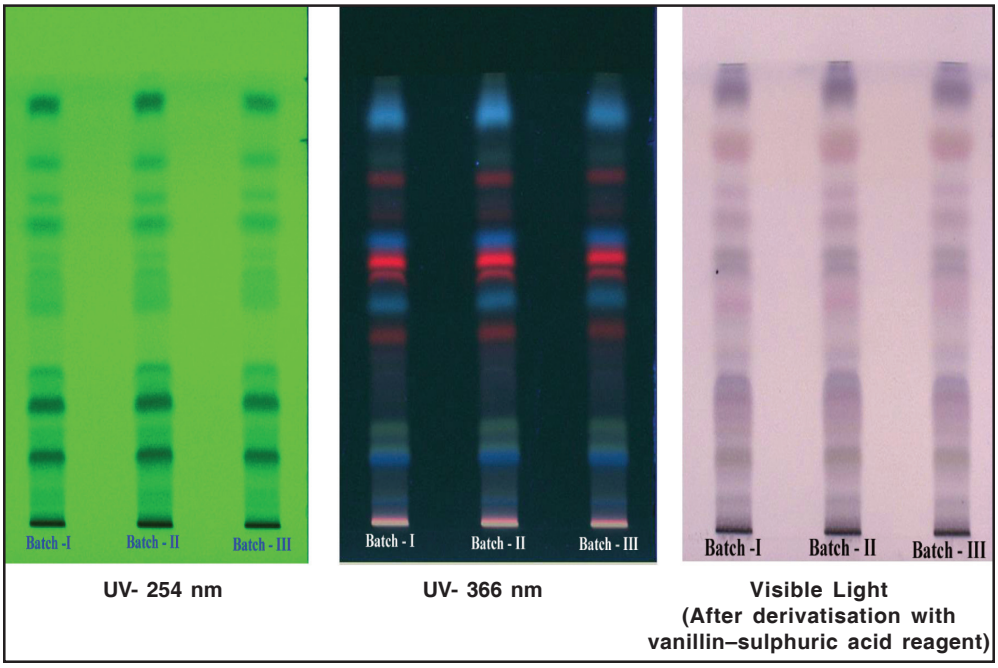

Fig. 3: TLC photos of chloroform extracts of three batch samples at different wavelength of light

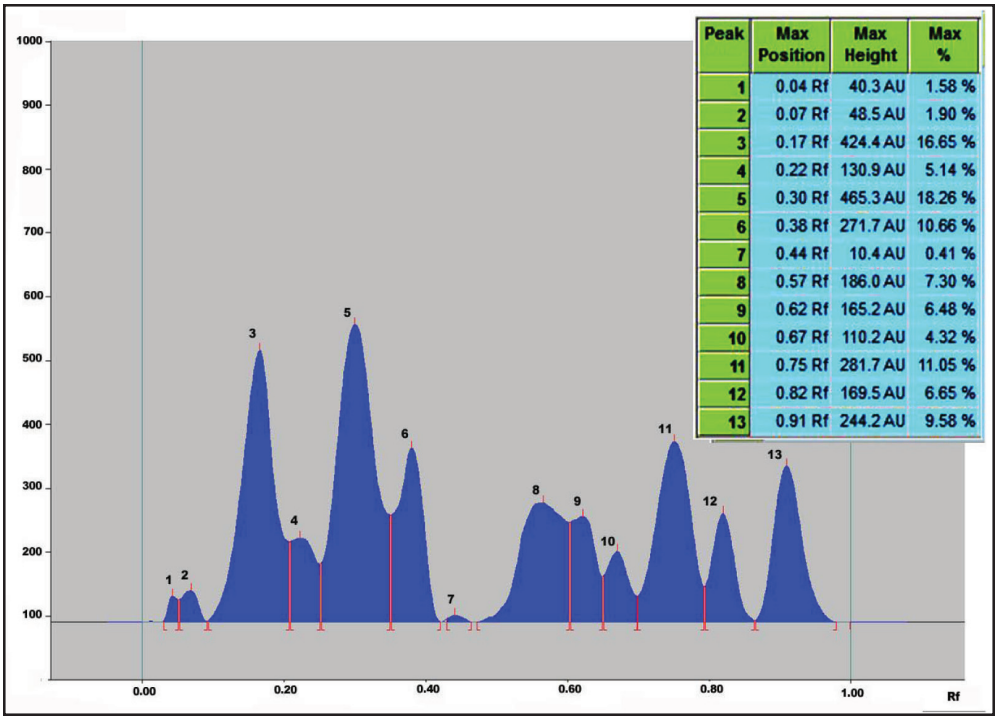

Fig. 4: HPTLC finger print profile for chloroform extract at 254 nm

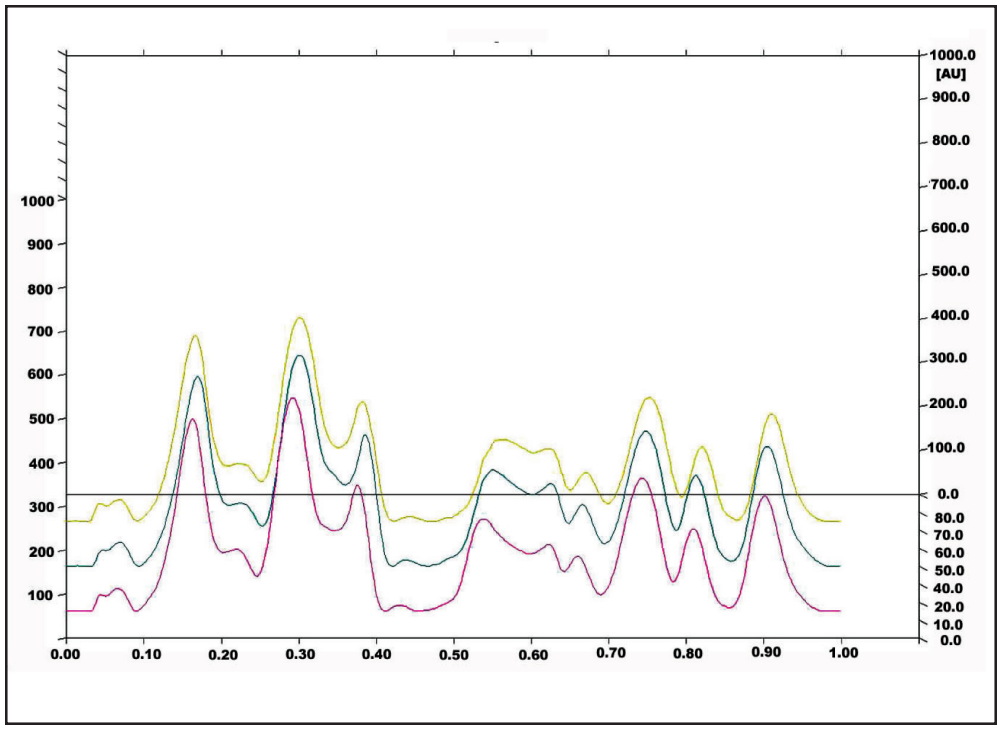

Fig. 5: HPTLC densitometry chromatogram of chloroform extracts of three batch samples at 254 nm

(ii) TLC/ HPTLC finger print studies of alcohol extract

The TLC studies of alcohol extract are tabulated in Table 4. All the three batch samples shows identical spot in UV-254 nm, UV-366 nm and visible light (after derivatised with vanillin – sulphuric acid reagent). In UV – 254, 366 nm and visible light it shows 6, 8 and 8 spots respectively with different R_f values (Fig. 6). The finger print of the alcohol extract shows 13 peaks of which peaks at R_f 0.54, 0.74, 0.81 and 0.90 were the major peak whereas peaks at R_f 0.08, 0.12, 0.25, 0.32, 0.37, 0.44, 0.60, 0.67 and 0.99 were moderately smaller peaks (Fig. 7). The HPTLC densitometry chromatogram of alcohol extract of three batch samples were recorded at 254 nm (Fig. 8).

Results and Discussion

The evaluated data in respect of powder microscopy, physico-chemical, TLC/ HPTLC fingerprint and other quality parameters provides analytical parameters on classical Unani formulation Jawarish-e-Kafoor Qawi can be used for in house quality control of drug as well for development of pharmacopoeial standards on drug.

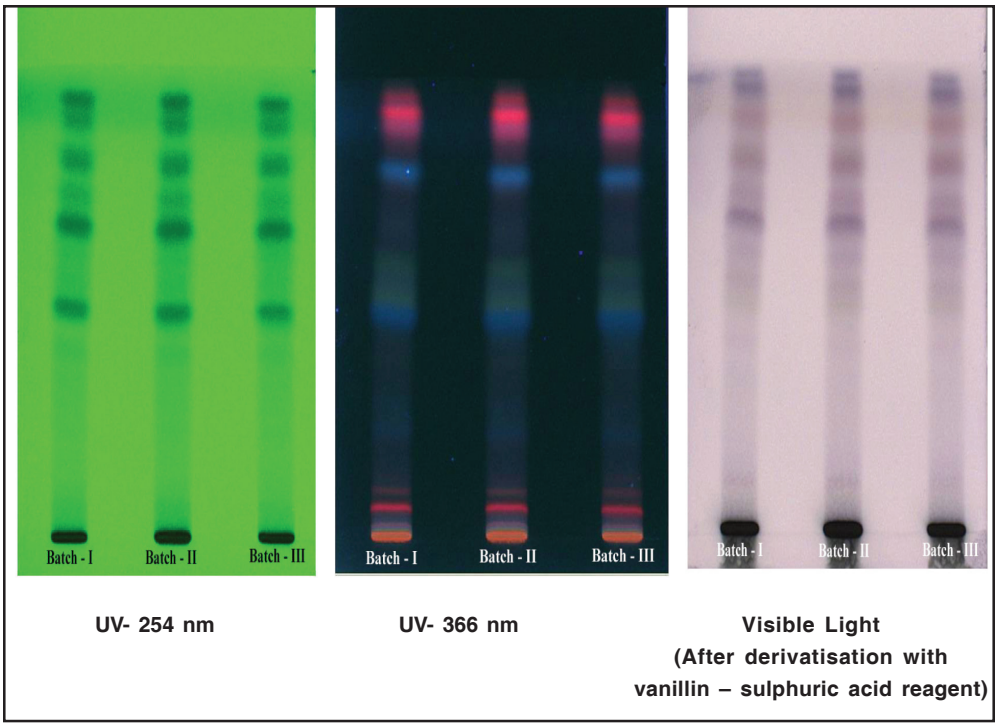

Fig. 6: TLC photos of alcohol extracts of three batch samples at different wavelength of light

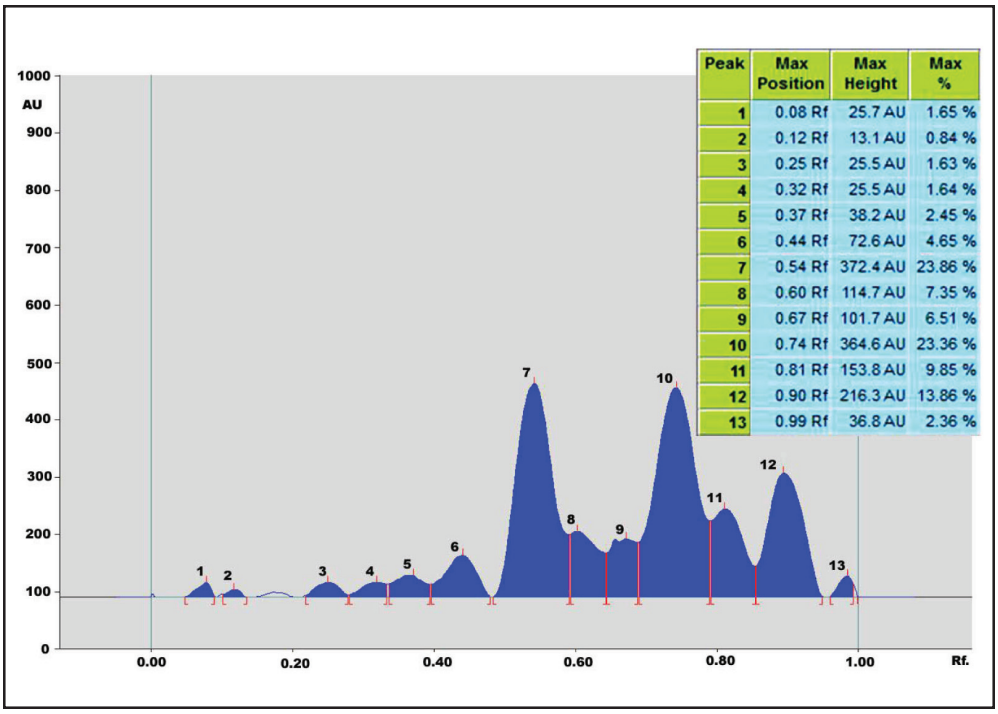

Fig. 7: HPTLC finger print profile for alcohol extract at 254 nm

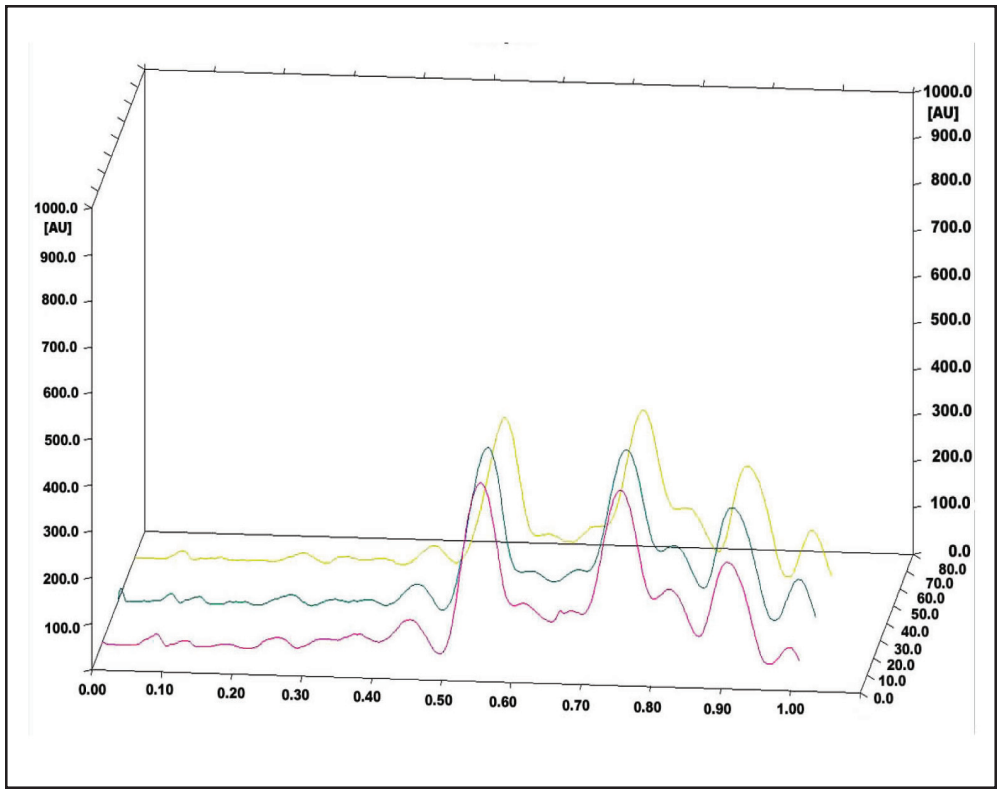

Fig. 8: HPTLC densitometry chromatogram of alcohol extracts of three batch samples at 254 nm

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