Standardization of *Laooq-E-Khiyarshambar*: A Classical Unani Formulation

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Abstract

Standardization of Unani herbal formulations is very much essential to justify the quality of a medicine. Unani medicines have played a significant role in maintaining of human health. These medicines are accepted as important therapeutic agents for the treatment of various kinds of diseases. But in many instances, it has been noticed that incorrect raw materials have been added in the formulations which has resulted adulterated product in market place. The Unani medicine Laooq-e-Khiyarshambar is an important polyherbal Unani medicine is being used in the ailments of catarrh, coryza, bronchitis and constipation. The drug Laooq-e-Khiyarshamber was standardized using standard methods such as pharmacognostical, physico-chemical and TLC/HPTLC. The other parameters like microbial load, heavy metals, aflatoxins and pesticide residues were also analyzed to ascertain the quality of medicine. The physico-chemical data such as moisture content was 19.23%. Alcohol soluble extractives 22.68% and water soluble extractive 69.61% shows presence of polar compound and inorganic material respectively. The content of total ash was 1.50% and acid insoluble ash 0.07% shows negligible amount of siliceous matter present in the drug. HPTLC finger print of chloroform and alcohol extracts shows13and 9 peaks with the developing systems toluene: ethyl acetate 8.5: 1.5and 6:4 respectively. The data evolved can be adopted for laying down the pharmacopoeial standards and TLC/HPTLC finger prints of the drug Laooq-e-Khiyarshamber.

Keywords: Laooq-e-Khiyarshambar, Powder microscopy, Physico-chemical, TLC/HPTLC and, WHO parameters

Introduction

Laooq-e-Khiyarshambar (Anonymous, 2006) is one of the classical Unani formulation commonly used in Unani System of Medicine for different kind of ailments. This classical poly-herbal formulation is prepared using 5 ingredients (Table–1). The Physicians of Unani System of Medicine prescribes this drug for the treatment of Nazla (Catarrh), Zukam (Coryza), Sual (Bronchitis) and Qabz (Constipation) disorders. Standardisation of Unani medicines is very much essential to provide safe, efficacious and quality product for the needy mass. Due to lack of scientific standards and standard protocol there are batch to batch variations in the same finished compound formulation. The main requirement of a standardisation is to establish the presence of each ingredient in the formulations (Bandaranayake WM, 2006; Myers SP and Cheras PA, 2004). The present study was aimed to evaluate the drug using modern parameters like

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Table 1: Ingredients Composition of Laooq-e-Khiyarshambar

S.No.	Unani Name	Botanical Names	Part Used	Quantity
1.	Sapistan API-VI	*Cordia dichotama* Forst.f.	Fruit	187.5g
2.	Asl-us-Soos UPI-I	*Glycyrrhiza glabra* Linn.	Dried root and Stolon	187.5g
3.	Maghz-e-Floos-e-Khiyarshambar UPI-I	*Cassia fistula* Linn	Fruit pulp	250g
4.	Kateera UPI-V I	*Cochlospermum religiosum* Linn.	Gum	125g
5.	Qand safaid	Sugar	—	3.5Kg

microscopical, physico-chemical, thin layer chromatography and WHO parameters viz., microbial load, aflatoxin, heavy metals and pesticide residue.

Material and Methods

To standardize the drug Laooq-e-Khiyarshambar a systematic scheme of standardization was followed.

Collection of the raw drugs

The raw drugs namely Sapistan, Asl-us-Soos, Maghz-e-Floos-e-Khiyarshambar, Kateera and Qand safaid used in the preparation of drug were procured from raw drug dealers of Chennai market. The raw drugs were identified using pharmacognostical methods and developed their pharmacopoeial standards.

Preparation of the drug

As per the ingredients composition and guidelines of NFUM, Part – I, this classical ploy- herbal drug Laooq-e-Khiyarshambar was prepared in different batches at Laboratory scale.

Powder microscopy

Microscopical examination reveals more information of a drug and helps to identify the organised drugs by their well known histological characters viz., cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibres and vessels (Kokate *et al*., 2000).

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. From this a few mg was taken in watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid and mounted in glycerine. The microscopic salient features of the drug were observed in different mounts (Wallis, 1997; Johansen, 1940).

Physico-chemical analysis

To standardise the drug physico-chemical methods viz., moisture content, ash values, solubility in different solvents, pH values, bulk density and sugar content etc., were used. The drug samples were subjected for the evaluation of physico-chemical and quality control parameters and analysed as per the standards method (Anonymous, 1987).

Thin layer chromatography

In Thin Layer Chromatographic method two phases were used for separation of phytoconstitues; one of these is a stationary phase bed and other is a mobile phase which percolates through this bed. TLC is the best method for recording the finger prints which can be reproduced anywhere at the same laboratory condition of a particular product.

The samples of the drug (2g) were soaked in chloroform and alcohol separately for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made up to 5ml in a standard flask separately and carried out the TLC studies (Wagner *et al*., 1984).

Quality control parameters

To justify the quality and higher safety margins, the WHO has taken necessary steps. In order to ensure the quality of a drug the modern suitable techniques and standard methods were adopted. The parameters such as microbial load and heavy metal were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxin and pesticide residues were carried out by standard methods (Anonymous, 2000).

Results and Discussion

Organoleptic character

The drug Laooq-e-Khiyarshambar is a blackish brown semi-solid formulation with sweetish bitter in taste.

Microscopical observation

The powder microscopic study of the drug Laooq-e-Khiyarshambar was evaluated and observed the salient features of the raw drugs used in the preparation and the photographs are shown in Fig. – 1.

Stone cells (Sclereids) lignified, thick walled upto 250µ with broad and narrow lumen, cotyledonary parenchyma cells in surface view filled with aleurone grains and oil globules (Sapistan); fragments of reticulate and pitted vessels, reticulate vessels upto 150µ, crystal sheath of parenchyma cells containing a prism of calcium oxalate crystals upto 25µ, cork cells in surface view, parenchyma cells filed with starch grins, starch grains simple spherical upto 25µ (Asl-us-Soos).

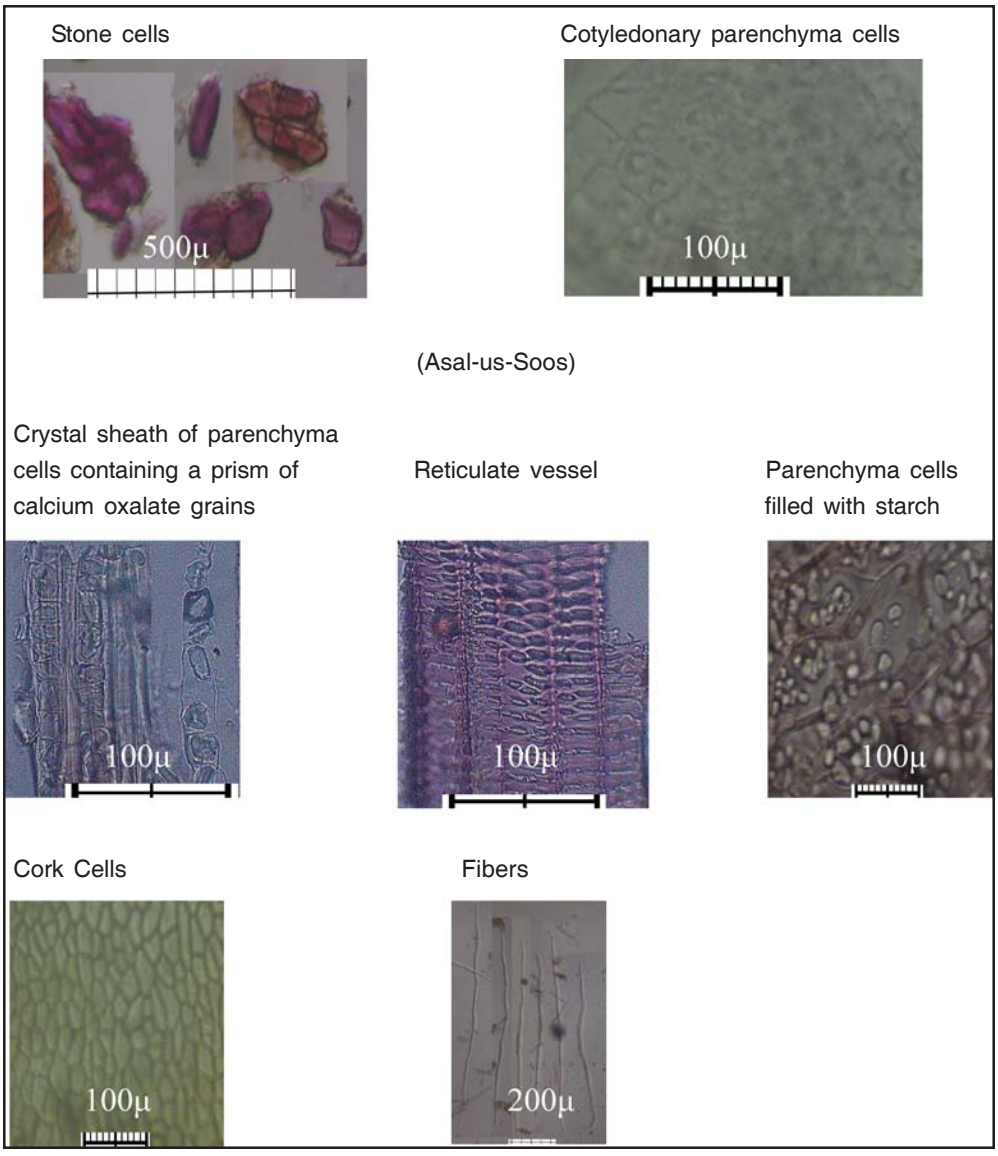


Fig. 1: Powder Microscopy (Sapistan)

Chemical analysis

The moisture content was obtained in the drug 19.23%. The physico-chemical data viz., alcohol soluble extractive (22.68%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (69.61%) indicate the presence of inorganic constituents. The obtained data are shown in Table-2.

Thin Layer Chromatography Analysis

The TLC studies of chloroform and alcohol extract of three batch samples were carried out and observed. All the three samples showed identical spots at UV – 254nm, 366nm and in VS Reagent (Fig. I, II & III). The R_f values of chloroform extracts shows major spots at 0.72, 0.63, 0.60, 0.48, 0.35, 0.24 and 0.10 (Green) in UV-254. Under UV (366nm), it shows major spots at R_f 0.95 (Pink), 0.86 (Blue), 0.78 (Light blue), 0.71, 0.64 (Blue), 0.57 (Fluorescent blue), 0.53 (Blue), 0.45, 0.41, 0.31 and 0.15 (Fluorescent blue). Then dipped the plate in vanillin-sulphuric acid reagent followed by heating at 110° about 5 min and observed under visible light, the plate shows major spots at R_f 0.87 (Grey), 0.72, 0.69, 0.61, 0.46 (Pink), 0.36, 0.22 (Violet) and 0.11 (Green).

Then applied the alcohol extracts on TLC plate and developed the plate using Toluene: Ethyl acetate (6: 4) as mobile phase. After development allowed the plate to dry in air and examine under UV (254nm), it shows major spots at R_f

Table 2: Physico-chemical parameters

Parameters Analyzed	Batch Number (n=3)		
	I	II	III
Extractives			
Alcohol soluble matter	22.73%	22.44%	22.88%
Water soluble matter	69.76%	69.44%	69.64%
Ash			
Total ash	1.39%	1.59%	1.54%
Acid insoluble ash	0.09%	0.06%	0.07%
pH values			
1% Aqueous solution	5.17	5.21	5.11
10% Aqueous solution	4.47	4.54	4.41
Sugar estimation			
Reducing sugar	17.25%	17.19%	17.31%
Non-reducing sugar	3.11%	3.05%	3.40%
Moisture	19.18%	19.31%	19.20%
Bulk Density	1.4705	1.4609	1.4809

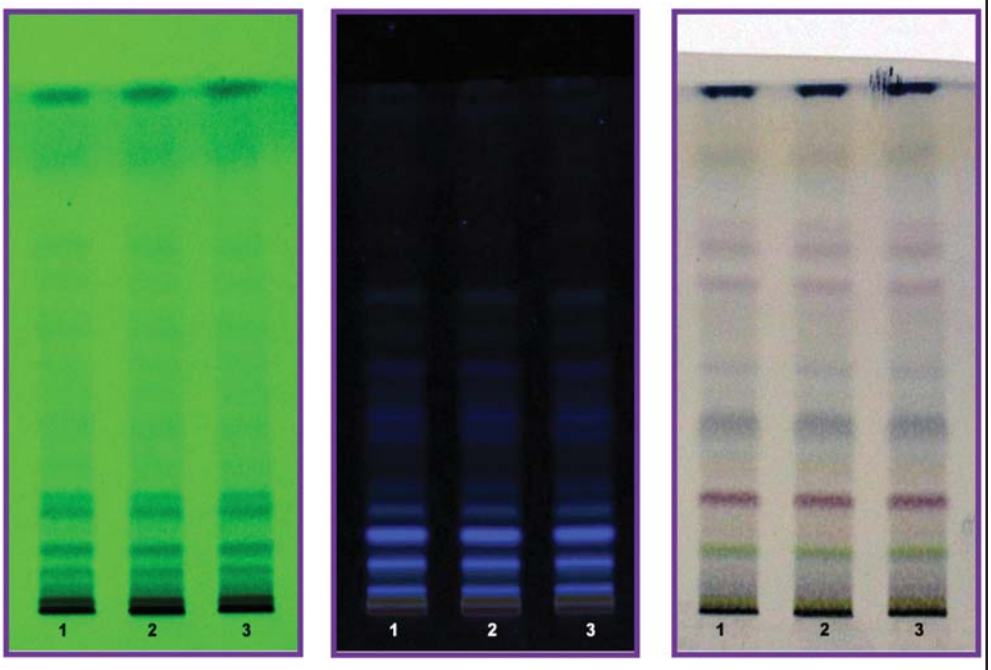
0.85, 0.68, 0.62, 0.53, 0.41, 0.34, 0.22, 0.18 and 0.12 (Green). Under UV (366nm), it shows major spots at R_f 0.96 (Pink), 0.86 (Fluorescent blue), 0.78, 0.70 (Blue), 0.62 (Fluorescent blue), 0.56 (Green), 0.52 (Blue), 0.45, 0.40, 0.30, 0.22 and 0.15 (Fluorescent blue). Dipped the plate in vanillin-sulphuric acid reagent followed by heating at 110° about 5 min and observed under visible light, the plate shows major spots at R_f 0.87 (Yellowish green), 0.72 (Light pink), 0.68, 0.62 (Pink), 0.45, 0.36 (Violet), 0.29 (Blue), 0.21 (Pink) and 0.15 (Green) (Fig.VI, VII & VIII).

Quality control parameters

The analyzed quality control parameters like microbial load and heavy metals were found within the permissible limit in the drug shown in Table - 3 and 4. 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 - 5 and 6.

Thin Layer Chromatography
(Chloroform Extract)

Fig.I Fig.II Fig.III



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

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

HPTLC finger print of chloroform extract

TLC plate was developed using Toluene: Ethyl acetate (8.5: 1.5) as mobile phase. After development allow the plate to dry in air, record the finger print (Fig. IV) and densitometric chromatogram (Fig .V) of the three batch samples of the compound formulation at 254 nm.

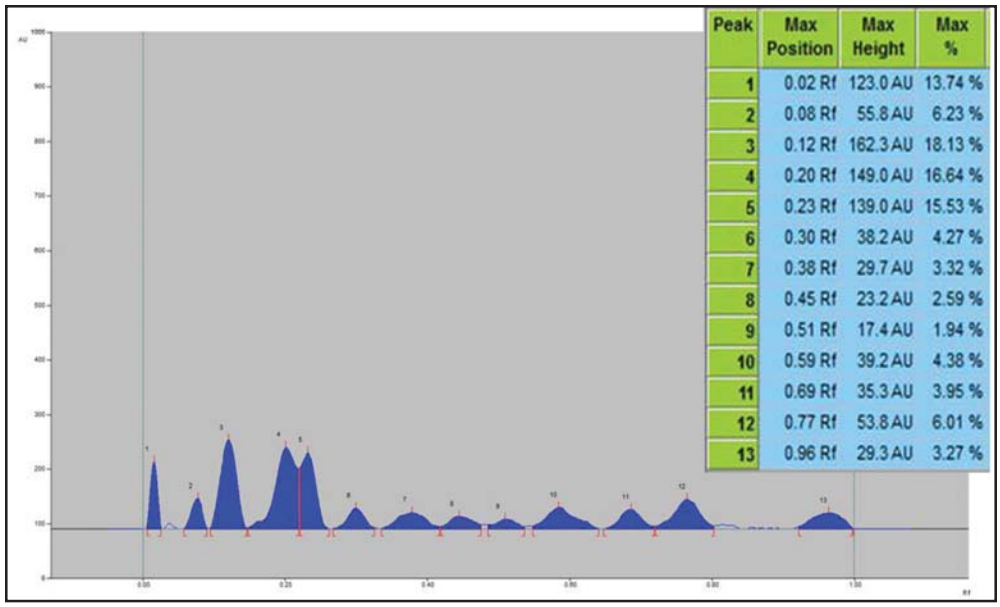


Fig. IV: HPTLC finger print of Laooq-e-Khiyarshambar chloroform extract at 254 nm

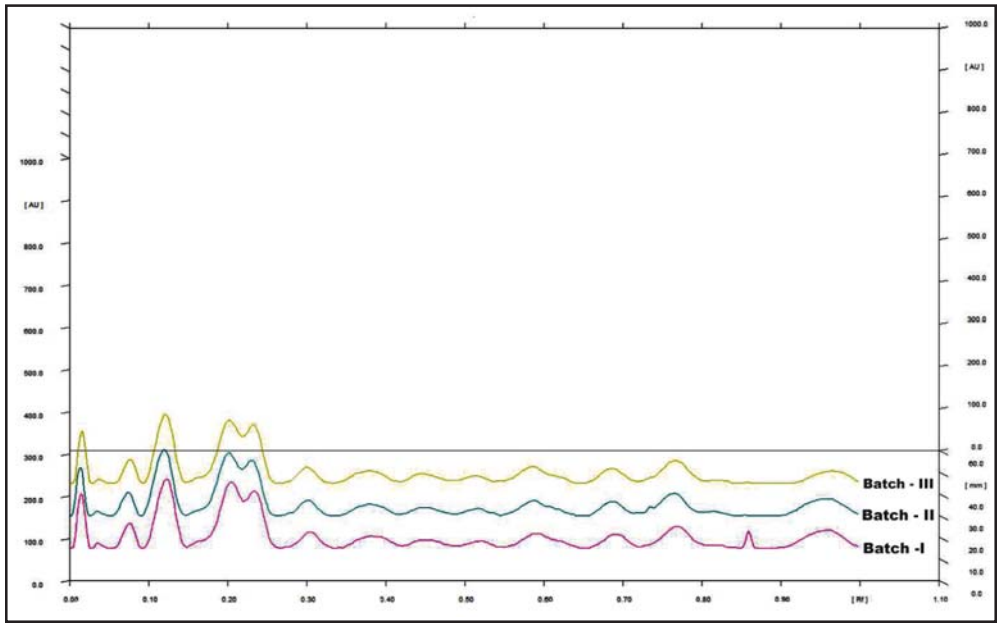
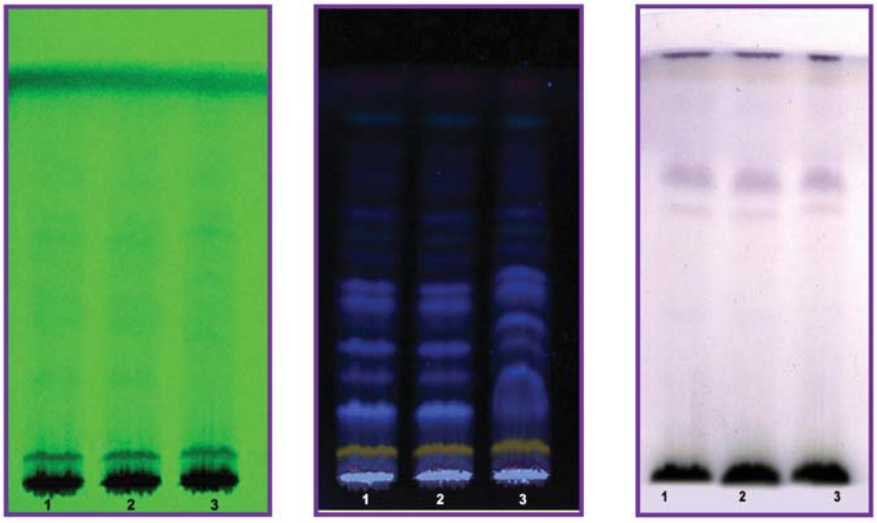


Fig. V: Densitometric chromatogram of Laooq-e-Khiyarshambar chloroform extracts at 254 nm

Thin Layer Chromatography
(Alcohol Extract)

Fig.VI Fig.VII Fig.VIII

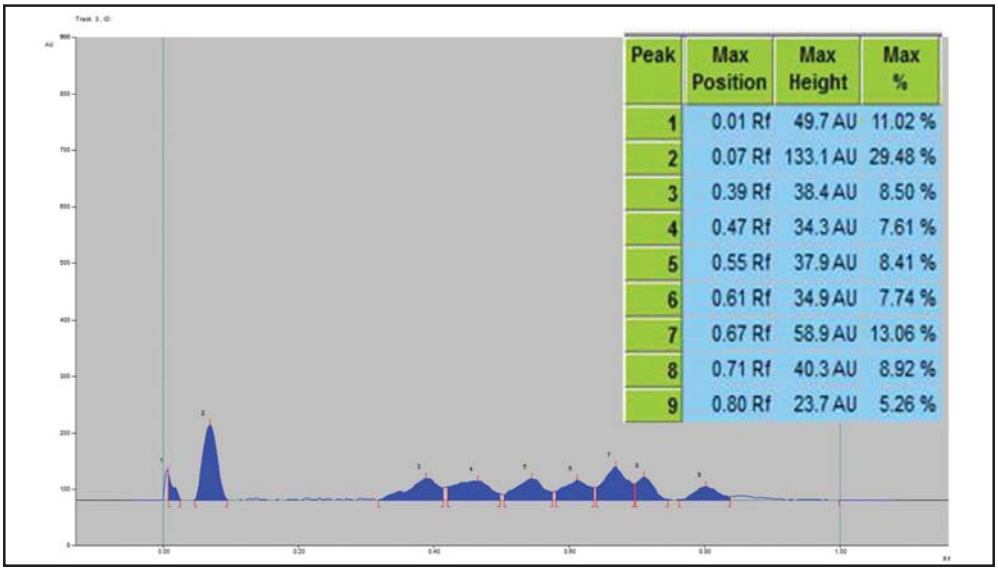


UV - 254 nm UV - 366 nm V - S Reagent

Solvent System : Toluene :Ethyl acetate (6:4)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch - III

HPTLC finger print of alcohol extract

TLC plate was developed using Toluene: Ethyl acetate (6: 4) as mobile phase. After development allow the plate to dry in air, record the finger print (Fig .IX) and densitometric chromatogram (Fig. X) of the three batch samples of the compound formulation at 254 nm.



Peak	Max Position	Max Height	Max %
1	0.01 Rf	49.7 AU	11.02 %
2	0.07 Rf	133.1 AU	29.48 %
3	0.39 Rf	38.4 AU	8.50 %
4	0.47 Rf	34.3 AU	7.61 %
5	0.55 Rf	37.9 AU	8.41 %
6	0.61 Rf	34.9 AU	7.74 %
7	0.67 Rf	58.9 AU	13.06 %
8	0.71 Rf	40.3 AU	8.92 %
9	0.80 Rf	23.7 AU	5.26 %

Fig.IX: HPTLC finger print of Laooq-e-Khiyarshambar alcohol extract at 254 nm

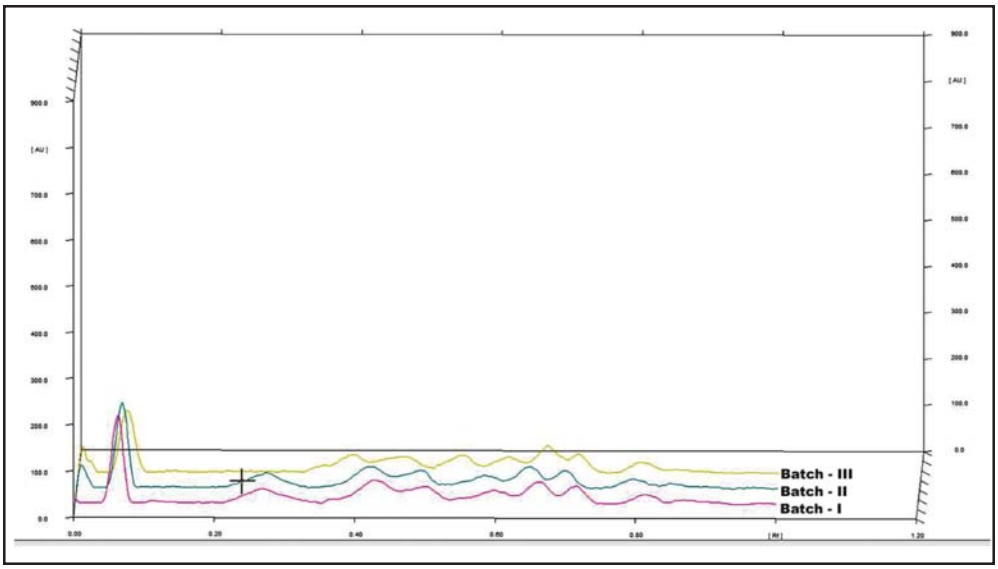


Fig. X: Densitometric chromatogram of Laooq-e-Khiyarshambar alcohol extracts at 254 nm

Table 3: Analysis of Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	7 x 10^3 CFU/gram	10^5 CFU / gm
2	Total Fungal Count	- Less than 10 CFU/gram	10^3 CFU / gm
3	Enterobacteriaceae	Absent / gm	10^3 CFU / gm
4	Salmonella	Absent / gm	Nil
5	*Staphylococcus aureus*	Absent / gm	Nil

Table 4: Estimation of Heavy Metals

S.No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Arsenic	Not detected	3 ppm
2	Cadmium	Not detected	0.3 ppm
3	Lead	0.0011	10 ppm
4	Mercury	Not detected	1.0 ppm

Table 5: Estimation of Aflatoxins

S.No.	Aflatoxins	Results	WHO Limits
1	B_1	ND	0. 5ppb
2	B_2	ND	0.1ppb
3	G_1	ND	0. 5ppb
4	G_2	ND	0.1ppb

ND = Not Detected

Table 6: 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

Conclusion

The evaluated data such as powder microscopy, physico-chemical, TLC/HPTLC fingerprints and analysis of quality control parameters indicates that the genuine raw drugs were added in the formulation and there is no variation in the batch to batch consistency of the drug.

Acknowledgement

The authors are extremely thankful to the Director General, CCRUM, New Delhi, for his valuable guidance, encouragement and providing necessary research facilities to carry out the present studies.

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