Pharmacopoeial Standardization of Unani Formulation Majoone-e-Lana

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Abstract

Standardization of herbal formulation is essential in order to assess the quality, purity, safety, and efficacy of drugs based on the analysis of their active properties. Testing of herbal preparations using scientific methodologies will add to quality and authenticity of the product. This article reports standardization parameters for Unani formulation Majoon-e- Lana used traditionally in the treatment of Muqawwi-e-Asab (nerve strengthening), Zof-e-Asab (neurasthenia), Falij (hemiplegia), Laqwa (facial paralysis), Rasha (tremor, trembling), Waj-ul-Mafasil (arthralgia) and Sara (epilepsy). Majoon-e-Lana is one of the Unani poly herbal formulations was prepared with the combination of twenty one ingredients as per National Formulary of Unani Medicine, and it was standardized by organoleptic characterization, physicochemical testing, thin layer chromatography/high performance thin layer chromatography, microbial load, heavy metal analysis, aflatoxins and pesticidal residues profiling employing a standard methodology. The physico-chemical data and TLC/HPTLC finger print analysis evolved can be adopted for laying down the pharmacopoeial standards for Majoon-e-Lana. All three different batch samples were found to be safe when tested for the heavy metal contamination, microbial load, aflatoxins and pesticide residues. Results of the experiments conducted provided diagnostic characteristics to identify and standardize the formulation prepared using ingredients of Majoon-e-Lana.

Keywords: Majoon-e-Lana, Physico-chemical parameters, TLC/HPTLC finger print, WHO parameters.

Introduction

Many modern medicines are directly or indirectly derived from higher plants (WHO, 2005). All medicines, whether synthetic or of plant origin, should fulfill the basic requirements of being safe and effective (EMEA, 2005; WHO, 2002). Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, and definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Quality of raw materials, good agricultural practices and good manufacturing practices play fundamental roles in guaranteeing the quality and stability of herbal preparations (WHO, 2000). Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence, standardization is a tool used in the quality control process (Kunle, 2012).

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Majoon-e-Lana is used in the ailments of Nerve strengthening, Neurasthenia, Hemiplegia, Facial paralysis, Tremor, Trembling, Arthralgia and Epilepsy. The present paper deals the physicochemical, TLC/HPTLC finger print, heavy metals, microbial load, aflatoxins and pesticide residues.

Materials and Methods

All the ingredients were procured from the local market and identified. Specimens of all ingredients of the formulation have been deposited in the museum of Drug Standardization Research Unit at Regional Research Institute of Unani Medicine, Chennai, Tamil Nadu, India. The drug Majoon-e-Lana was prepared as per the formulation composition given in NFUM, Part-I using 21 ingredients showed in table 1 (Anonymous, 1981).

Table 1: List of ingredients of Majoon-e-Lana

Azaraqi Mudabhar	Strychnos nux-vomica Linn.	Seed	20g
Filfil Safaid	*Piper nigrum* Linn.	Fruit	10g
Filfil Siyah	*Piper nigrum* Linn.	Fruit	10g
Darchini	*Cinnamomum zeylanicum* Blume.	Inner stem bark	10g
Filfil Daraz	*Piper longum* Linn.	Fruit	10g
Jauzbuwa	*Myristica fragrans* Houtt.	Endosperm	10g
Bisbasa	*Myristica fragrans* Houtt.	Arillus	10g
Mastagi	*Pistacia lentisus* Linn.	Resin	10g
Sad Kufi	*Cyperus rotundus* Linn.	Rhizome	10g
Zanjabeel	*Zingiber officianale* Rosc.	Rhizome	10g
Qaranful	*Syzygium aromaticum* Merr & L.M. Perry	Flower bud	10g
Aamla	*Emblica officinalis* Gaertn.	Fruit	10g
Sumul-ut-Teeb	*Nardostachys jatamansi* Dc.	Rhizome	10g
Heel Khurd	*Elettaria cardamomum* Maton.	Fruit	10g
Nankhwah	*Trachyspermum ammi* (L.) Sprague. ex Turril	Fruit	10g
Badiyan	*Foeniculum vulgare* Mill.	Fruit	10g
Zafran	*Crocus sativus* Linn.	Stamen & Stigma	10g
Sandal Safaid	*Santalum album* Linn.	Heart wood	10g
Ood-e-Balsan	*Commiphora opobalsamum* (L.) Engl.	Wood	10g
Agar	*Aquilaria agallocha* Roxb.	Heart wood	10g
Qand Safaid	Sugar	—	600g

Method of preparation of the drug

All the ingredients were taken of pharmacopoeial quality. Cleaned, dried, powdered and sieved through 80 mesh. Mixed the powders of all the ingredients of Azaraqi Mudabhar, Filfil Safaid, Filfil Siyah, Darchini, Filfil Daraz, Jauzbuwa, Bisbasa, Mastagi, Sad Kufi, Zanjabeel, Qaranful, Aamla, Sumul-ut-Teeb, Heel Khurd, Nankhwah, Badiyan, Zafran, Sandal Safaid, Ood-e-Balsan and Agar, kept separately. Dissolved the specified quantity of ingredient Qand Safaid on slow heat in 600 ml of water, at the boiling stage added 0.1% citric acid and mixed thoroughly. At the stage of 70% consistencies of quiwam, 0.1% sodium benzoate was added and mixed thoroughly to prepare the quiwam of 76% consistency. Removed the vessel from the fire, while hot condition the mixed powders of all the ingredients were added and mixed thoroughly to prepare the homogenous product. Allowed it to cool to room temperature and packed in tightly closed containers to protect from light and moisture.

Physico-chemical analysis

The analytical data like moisture content, ash values, alcohol and water soluble extractives, pH values, bulk density and estimation of sugar were arrived by employing the standard procedure (Anonymous, 1998 and Anonymous, 1987).

TLC/HPTLC finger print analysis

Preparation of extracts for TLC

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 Linomat IV sample 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 and observed the spots at UV-254 nm, UV-366 nm and the plates were scanned at 254 nm to record the finger print spectrum. Finally the plate were dipped in vanillin-sulphuric acid and heated at 105° C till coloured spots appeared (Wagner and Bladt, 1984; Sethi, 1996).

Estimation of microbial load

The estimation of microbial load viz. total bacterial count (TBC), total fungal count (TFC), Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* were determined as per WHO standards (1998).

Estimation of Heavy Metals

The procedure was used for the analysis of heavy metals like lead, cadmium, mercury and arsenic as per WHO, 1998 and AOAC, 2005.

Instrument details and operating parameters

Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis. The operating parameters:

Lead and Cadmium: Instrument technique - Flame technique; wavelength (Lead) - 217 nm; wavelength (Cadmium) - 228.8 nm; slit width - 0. 5 mm; lamp current (Pb) - 4.0 mA; lamp current (Cd) - 3.0 mA; carrier gas and flow rate - air and acetylene, 1.1 L/min; sample flow rate - 2 ml/min. Mercury: Instrument technique - Cold vapour technique; wavelength - 253.7 nm; slit width - 0. 5 mm; lamp current - 3.0 mA; carrier gas and flow rate - argon, 1.1 L/min; sample flow rate - 5ml/min. Arsenic: Instrument technique - Flame vapour technique; wavelength - 193.7 nm; slit width - 0. 5 mm; lamp current - 6.0 mA; carrier gas and flow rate - acetylene, argon, 1.1 L/min; sample flow rate - 5ml/min. The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

Analysis of Aflatoxins

The procedure was followed for the analysis of aflatoxins B_1, B_2, G_1 and G_2 as per Official Analytical Methods of the American Spice Trade Association (ASTA) (1997).

Instrument details and operating parameters

Thermo Fisher High Performance Liquid Chromatography (HPLC) was used for the aflatoxins analysis. Column - Ultra C18, 250 X 4.6 mm, 5 µm particles; mobile phase - water: acetonitrile: methanol (65: 22.5: 22.5); flow rate - 1 ml/min; temperature - 35º C; detector - fluorescence detector at 360 nm; injection - 20 µl (Aflatoxins mixture and sample)

Analysis of pesticide residue

The procedure was followed for the analysis of pesticidal residues as per AOAC, 2005. Pesticidal residues were analyzed by Gas Chromatography-Mass Spectra

(GC-MS)(Instrument-Agilent, detector-mass selective detector, column specification-DB5MS, carrier gas- helium, flow rate-1ml/min, column length- 30 m, internal diameter-0.25 mm, column thickness-0.25 μm).

Results and Discussion

The drug is brown in colour, semi-solid, characteristic of its own odour and sweetish bitter in taste.

Physico-chemical parameters

Physico-chemical parameters of Majoon-e-Lana are tabulated in Table-2. Quantitative standards revealed that the moisture content was 19.65%, ash content was 1.37% and acid insoluble ash 0.46% indicates the negligible amount of siliceous matter present in the drug. The water soluble extractive value of the drug 66.36% indicates the presence of inorganic content and the alcohol soluble extractive value 34.53% indicates the extraction of polar constituents.

Table 2: Physico-chemical parameters of the Majoon-e-Lana

S.No.	Parameters	Majoon-e-Lana		
		Batch-I	Batch-II	Batch-III
1	Moisture (% w/w)	19.44	19.78	19.74
2	Extractive values (% w/w)			
	Alcohol soluble matter	34.56	34.75	34.28
	Water soluble matter	66.14	66.40	66.56
3	Ash values (% w/w)			
	Total ash	1.62	1.28	1.23
	Acid insoluble ash	0.60	0.43	0.37
4	*pH* values			
	1% Aqueous solution	5.59	5.42	5.27
	10% Aqueous solution	4.65	4.51	4.42
5	Sugar estimation			
	Reducing sugar (% w/w)	41.40	41.34	41.55
	Non reducing sugar (% w/w)	9.42	9.36	9.49
6	Bulk Density	1.6509	1.6501	1.6405

All values are mean of three determinations

TLC studies of chloroform extract

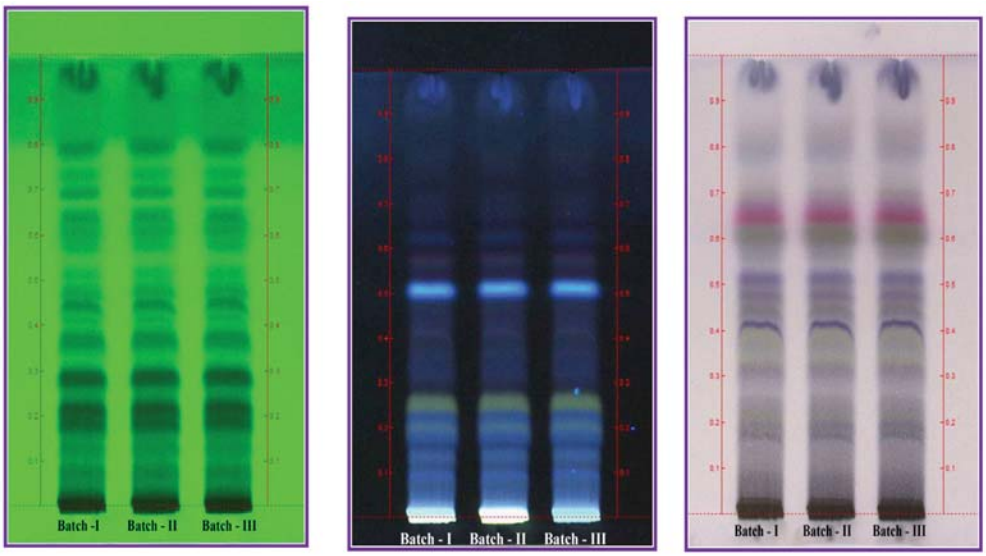
The TLC studies of chloroform extract are tabulated in Table - 3. All the three batch samples showed identical spots in UV-254 nm, UV-366 nm and visible light (after derivatised with vanillin – sulphuric acid reagent). In UV – 254 nm, 366 nm and visible light it shows 15, 10 and 12 spots respectively with different R_f values (Fig. 1).

HPTLC finger print studies of chloroform extract

The finger print of the chloroform extract shows 15 peaks of which peaks at R_f 0.24, 0.32, 0.42, 0.52, 0.70, 0.73, 0.80 and 0.92 were the major peak whereas peaks at R_f 0.02, 0.07, 0.14, 0.47, 0.54 and 0.85 were moderately smaller peaks (Fig.2). The HPTLC densitometry chromatogram of chloroform extract of three batch samples were found to be same when scanned at 254 nm (Fig. 3).

Table 3: R_f values of the chloroform extract

Solvent System	*Rf* Values		
	UV- 254 nm	**UV – 366 nm**	**Visible light (after derivatization with vanillin – sulphuric acid reagent)**
Toluene: Ethyl acetate (9:1)	0.79 Green	0.80 Light blue	0.84 Brown
	0.74 Green	0.68 Light blue	0.79 Violet
	0.70 Green	0.63 Blue	0.70 Grey
	0.65 Green	0.57 Blue	0.65 Red
	0.61 Green	0.51 Fluorescent blue	0.61 Grey
	0.58 Green	0.41 Blue	0.52 Violet
	0.52 Green	0.26 Yellowish green	0.48 Violet
	0.48 Green	0.22 Violet	0.45 Grey
	0.45 Green	0.18 Blue	0.42 Violet
	0.41 Green	0.14 Blue	0.32 Grey
	0.37 Green		0.24 Grey
	0.29 Green		0.19 Violet
	0.22 Green		
	0.18 Green		
	0.13 Green		



UV- 254 nm | UV- 366 nm | Visible Light (After derivatisation with vanillin – sulphuric acid reagent)

Fig. 1: TLC photos of chloroform extracts of three batch samples

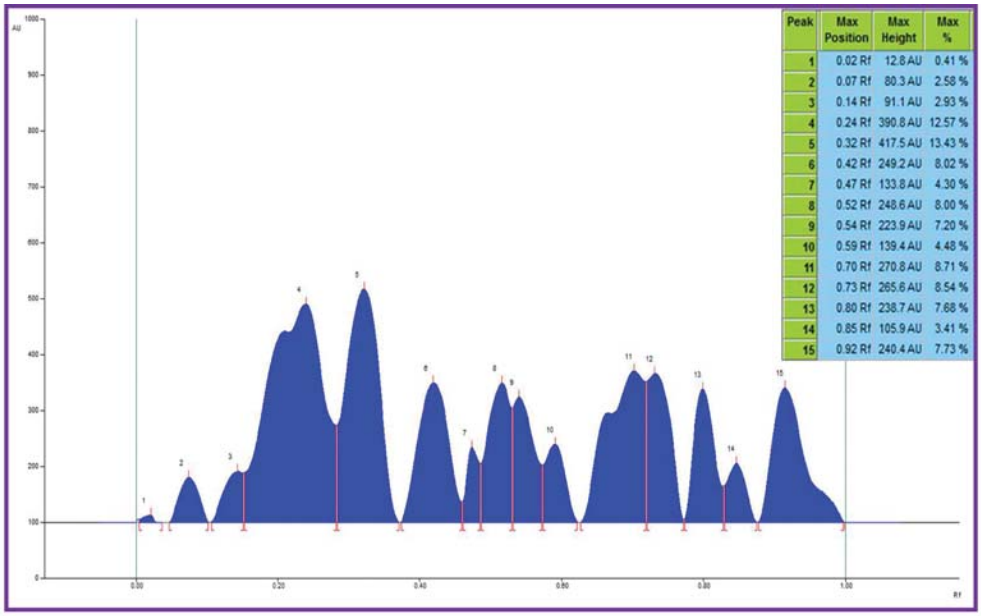


Fig. 2: HPTLC finger print profile of chloroform extract at 254 nm

TLC studies of alcohol extract

The TLC studies of alcohol extract are tabulated in Table - 4. All the three batch samples showed identical spot in UV-254 nm, UV-366 nm and visible light (after

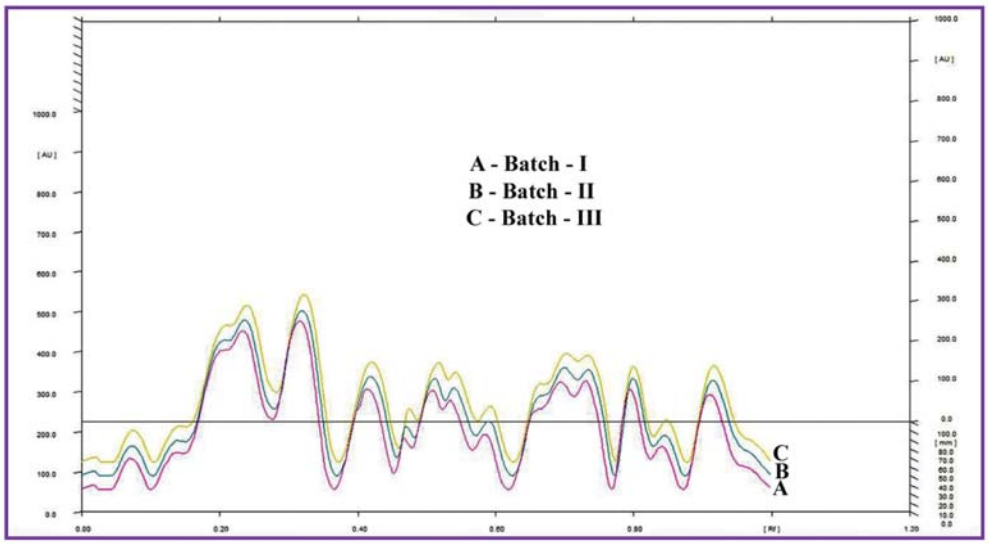
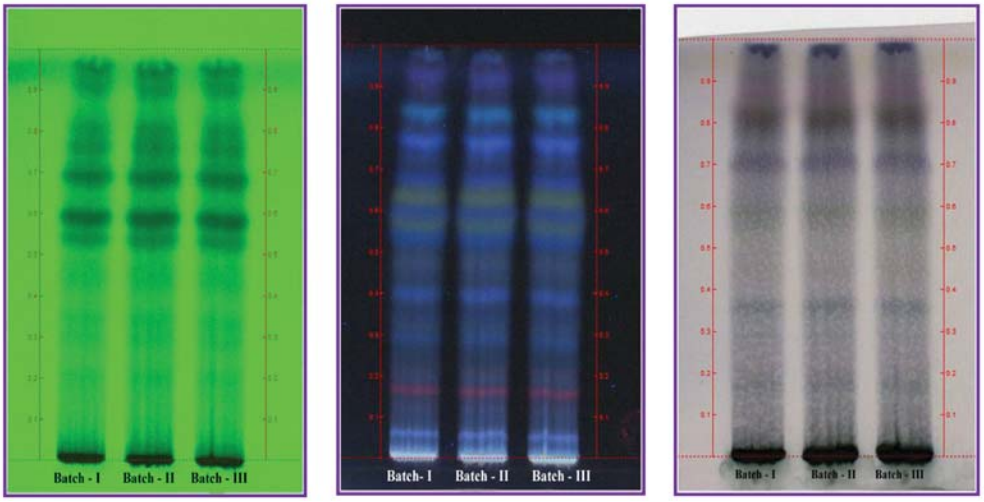


Fig. 3: HPTLC densitometry chromatogram of chloroform extracts of three batch samples at 254 nm

derivatised with vanillin – sulphuric acid reagent). In UV – 254 nm, 366 nm and visible light it shows 10, 13 and 7 spots respectively with different R_f values (Fig.4).

Table 4: R_f values of the alcohol extract

Solvent System	R_f Values		
	UV- 254 nm	UV – 366 nm	Visible Light (After derivatisation with vanillin – sulphuric acid reagent)
Toluene: Ethyl acetate (6:4)	0.90 Green	0.92 Fluorescent blue	0.87 Pink
	0.81 Green	0.83 Fluorescent blue	0.82 Grey
	0.76 Green	0.77 Fluorescent blue	0.71 Violet
	0.69 Green	0.67 Blue	0.58 Grey
	0.59 Green	0.63 Yellowish green	0.36 Blue
	0.53 Green	0.59 Blue	0.29 Grey
	0.44 Green	0.56 Yellowish green	0.18 Blue
	0.35 Green	0.53 Blue	
	0.28 Green	0.46 Blue	
	0.20 Green	0.39 Fluorescent blue	
		0.29 Blue	
		0.20 Brown	
		0.16 Red	



UV- 254 nm | UV- 366 nm | Visible Light (After derivatisation with vanillin – sulphuric acid reagent)

Fig. 4. TLC photos of alcohol extracts of three batch samples

HPTLC finger print studies of alcohol extract

The finger print of the chloroform extract shows 13 peaks of which peaks at R_f 0.63, 0.69, 0.81 and 0.89 were the major peak whereas peaks at R_f 0.04, 0.24, 0.34, 0.40, 0.52 and 0.94 were moderately smaller peaks (Fig. 5). The HPTLC densitometry chromatogram of alcohol extract of three batch samples were found to be same when scanned at 254 nm (Fig. 6).

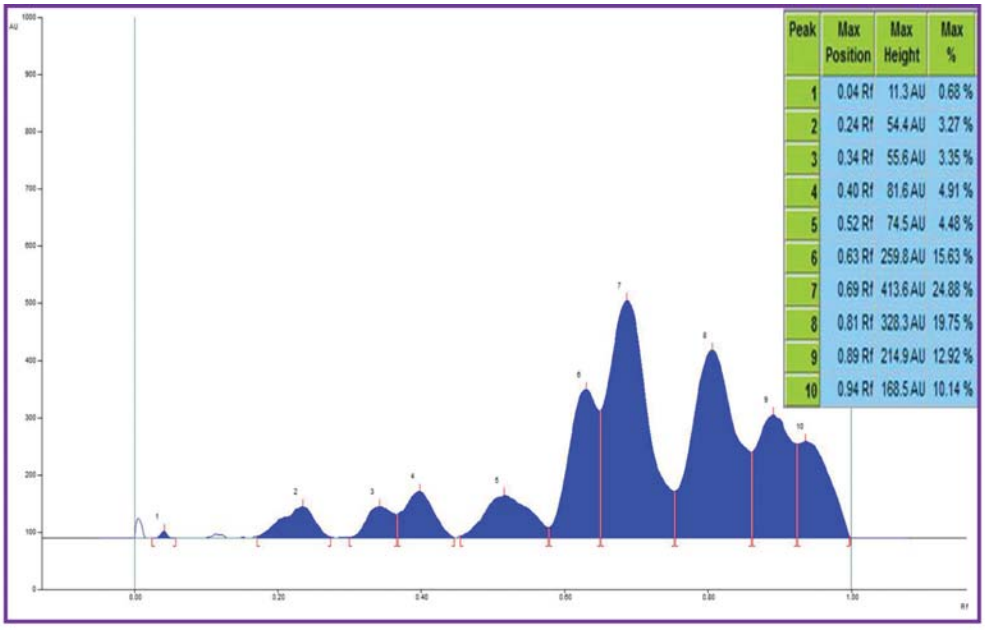


Fig. 5: HPTLC finger print profile of alcohol extract at 254 nm

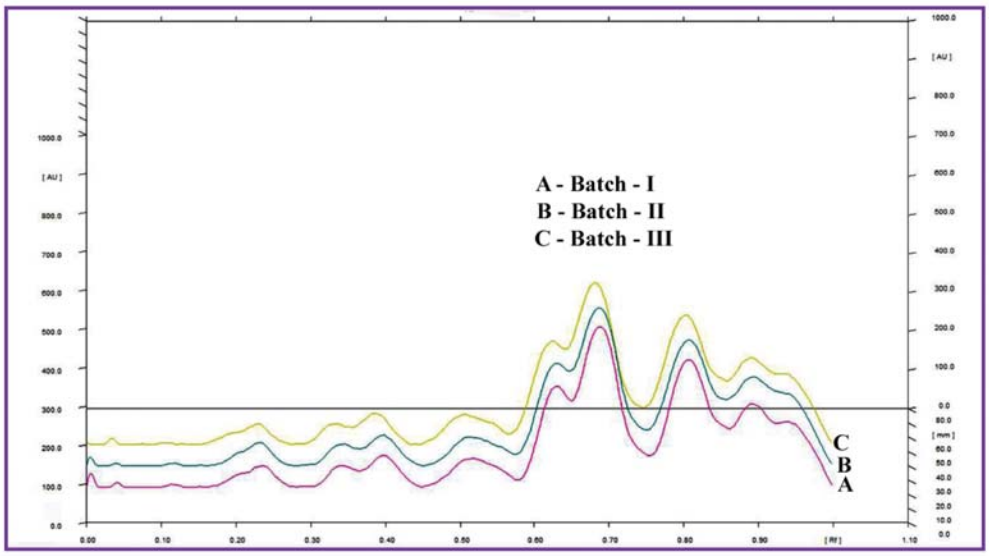


Fig. 6: HPTLC densitometry chromatogram of alcohol extracts of three batch samples at 254 nm

Microbial load, Heavy Metals, Aflatoxins and Pesticidal residues

Estimation of microbial load viz. Total bacterial count (TBC), Total fungal count (TFC), Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* were found to be within the permissible limit as stated by WHO (Table 5). The heavy metals viz. lead was present within the permissible limit where as cadmium; mercury and arsenic were not found in the drug (Table 6). The studies of other parameters like estimation of afltoxins such as B_1, B_2, G_1 and G_2 and pesticide residue such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion were not detected from the drug.

Table 5: Microbial Load

Parameters	Results	WHO Limits for internal use
Total Bacterial Count (TBC)	3 x 10^2 cfu/gram	1x10^5cfu/g
Total Fungal Count (TFC)	Less than 10 cfu/gram	1x10^3cfu/g
Enterobacteriaceae	Absent	1x10^3cfu/g
Escherichia coli	Absent	1x10^1cfu/g
Salmonella spp	Absent	Absent
Staphylococcus aureus	Absent	Absent

Table 6: Analysis of Heavy Metals

Sl.No	Parameters	Values
1.	Lead	0.0128 ppm
2.	Cadmium	Not detected
3.	Arsenic	Not detected
4.	Mercury	Not detected

All values are mean of three determinations

Conclusion

Standardization is an important aspect of any herbal formulation development. It is important to identify and record the physical, physicochemical and chemical properties of each plant material that is involved in product development of Majoon-e-Lana to maintain the batch-to-batch consistency and quality of the products. The physic-chemical parameters will be helpful for fixing pharmacopoeial standards of the drug. TLC/HPTLC finger print profile of chloroform and alcohol extracts provides a suitable method for monitoring the identity and purity and also standardization of the drug. Heavy metals, aflatoxins, pesticidal residues and microbial load were found to be within the permissible limit of WHO, indicating that the drug is free from toxic materials and which can be used in the ailments of stomachic, digestive and brain disorders.

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 studies.

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