# Scientific Validation of Unani Formulation: 'Majoon-e-Hamal Ambari Alwi Khani'

[1*]Rampratap Meena,
[2]S.C. Verma,
[3]S. Mageswari,
[3]P. Meera Devi Sri,
[1]A.S. Khan,
[1]S.A. Ansari,
[4]Shamsul Arfin
and
[4]Aminuddin

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

[2]Pharmacopoeia Commission of Indian Medicine & Homoeopathy, PLIM Campus, Ghaziabad-201002

[3]Regional Research Institute of Unani Medicine, 1 West Mada Church Street, Chennai-600013

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

## Abstract

The quality and safety of the Unani formulations have to be scientifically validated in order to produce quality medicines and protect the health of people suffering from various kinds of diseases. For the validation or standardization of Unani formulations a systematic scientific protocol is very much needed. Though the Unani medicines are an important healthcare option, but due to lack of scientific standards, these traditional medicines are hindering us from capitalizing on the global opportunities provided by increasing interests all over the world. The drug 'Majoon-e-Hamal Ambari Alwi Khani' is one of the ancient most commonly used Unani formulations in the ailments of atony to the uterus and habitual abortion. For the validation of this drug the parameters such as Standard Operating Procedure (SOP's) for the preparation of drug, powder microscopy to find out the characteristic botanical characters of raw drugs added in the formulation were worked out. Evaluation of physico-chemical, TLC/HPTLC, finger prints, analysis of WHO parameters like heavy metals, microbial loads, aflotoxins and pesticide residues were also carried out to ascertain the quality of drug. The evaluated data will help to lay down the scientific standards for the drug studied to contribute mateial for in Unani pharmacopoeia of India.

**Keywords:** 'Majoon-e-Hamal Ambari Alwi Khani', Microscopy, TLC/HPTLC, Scientific validation.

## Introduction

Plenty of modern medicines are derived from higher plants (Anonymous, 2005). All medicines, either synthetic or plant origin, have to fulfill the basic requirements of safety and efficacy (EMEA, 2005; Anonymous, 2002). Standardization of traditional medicines is the process of evaluation of a set of pharmacopoeial standards and definitive qualitative and quantitative values which gives an assurance of quality, efficacy, safety and reproducibility. Quality of raw materials, good laboratory practices and good manufacturing processes plays the important roles for providing the quality and efficacious herbal preparations for the needy mass (Anonymous, 2000). Validation of pharmacopoeial standards by experimentation and observations provides a set of characteristics to a particular herbal medicine. Therefore, Scientific Validation of Unani Formulations is an important tool used in the standardization process (Kunle, 2012). Majoon-e-Hamal Ambari Alwi Khani is used in the ailments of Atony to the Uterus and Habitual abortion. Present research studies deals the pharmacognostical, physicochemical, TLC/HPTLC finger print, heavy metals, microbial load, aflatoxins and pesticide residues.

[1*]Author for correspondence

## Materials and Methods

All the ingredients were procured from the local market and identified. Specimens of all ingredients of the formulation were deposited in the museum of Drug Standardization Research Unit at Regional Research Institute of Unani Medicine, Chennai, Tamil Nadu, India The drug Majoon-e-Hamal Ambari Alwi Khani was prepared as per the formulation composition given in NFUM, Part-I using 20 ingredients showed in Table 1 (Anonymous, 1981).

**Table 1:** Formulation composition of 'Majoon-e-Hamal Ambari Alwi Khani'

| | | | | |
|---|---|---|---|---|
| 1. | Ambar Ash-hab | *Ambra grasea* (Animal origin) | Part Used | 30g |
| 2. | Tukhm-e-Khurfa | *Portulaca oleracea* Linn. | Seed | 25g |
| 3. | Maghz-e-Tukhm-e-Petha | *Benincasa hispida* (Thunb.) Cogn. | Kernel | 25g |
| 4. | Marwarreed | *Mytilus margaritiferus* (Animal origin) | Pearl | 10g |
| 5. | Kahruba | *Pinus succinifera* Linn. | Resin | 10g |
| 6. | Busud Mohraq | *Corallium ruburum* (Animal origin) | Coral roots | 10g |
| 7. | Sandal Surkh UPI-V | *Pterocarpus santalinus* Linn. | Heart wood | 10g |
| 8. | Sandal Safaid UPI-VI | *Santalum album* Linn. | Heart wood | 10g |
| 9. | Tabasheer | *Bambusa bambos* Druce. | Bamboo Manna | 10g |
| 10. | Mazu UPI-III | *Quercus infectoria* Oliv. | Excrescence/ Gall | 10g |
| 11. | Darunaj Aqrabi API-VI | *Doronicum hookeri* Hook.f. | Rhizome | 10g |
| 12. | Ood-e-Saleeb UPI-III | *Paeonia emodi* Wall. | Tuber | 10g |
| 13. | Abresham | Bombyx mori (Animal origin) | Silk Cocoon | 10g |
| 14. | Beikh-e-Anjabar UPI-III | *Polygonum bestorta* Linn. | Rhizome | 10g |
| 15. | Gil-e-Armani | Silicate of Alumina, Magnesia and oxide of Iron | — | 10g |
| 16. | Sharbat-e-Angoor | *Vitis vinefera* Linn. | Fruit | 400 ml |
| 17. | Waraq-e-Nuqra | Gold leaf | — | 20 Nos. |
| 18. | Waraq-e-Tila | Silver leaf | — | 20 Nos. |
| 19. | Asal | Honey | — | 200g |
| 20. | Qand Safaid | Sugar | — | 700g |

## Method of Preparation

All the ingredients were taken of pharmacopoeial quality. Grinded the ingredient number 1 of the formulation composition in mortar & pestle and kept separately. Cleaned, dried and powdered the ingredient number 2 to 3, 5 to 12, 14 and 15 of the formulation composition and sieved through 80 meshes. Made the coarse powder of ingredient number 4 of the formulation composition and soaked in Arq-e-Gulab for 5 days and grinded it daily about 4 hours using mortar and pestle till becomes nice powder. Dissolved the ingredient number 20 in 800 ml of water on slow heat, at the boiling stage 0.1% citric acid was added and mixed thoroughly. Boiled the content and prepared the quiwam of 72% consistency and added 0.1% sodium benzoate. At this stage added the ingredient number 16, 19 and extract of ingredient number 13, mixed well and made the quiwam of 76% consistency.

Then vessel was removed from the fire, while hot condition ingredient number 1 was added and mixed well, finally mixed powders of ingredient number 2 to 12, 14 and 15 were added, mixed thoroughly and obtained the homogenous product.

Allowed it to cool to room temperature and added the required quantity of ingredient number 17 and 18 and mixed thoroughly and was packed in tightly closed containers to protect from light and moisture.

## Powder Microscopy

Drug (5g) was weighed, mixed with 50ml of water in a beaker and warmed gently inorder to make complete dispersion in water. Then mixture was centrifuged and decanted supernatant. The sediment was washed several times with distilled water, centrifuged again and decanted the supernatant. Small quantity of the sediment was taken and mounted in glycerin, out of which another small quantity was taken in watch glass and a few drops of phloroglucinol and concentrated hydrochloric acid were added, mounted in glycerin to locate lignified cells. The following characters in different mounts were observed (Wallis, 1987; Johansen, 1940).

## Physico-chemical Analysis

The Physico-chemical data viz., moisture content, ash values, alcohol and water soluble extractives, pH value, bulk density and estimation of sugar were evaluated as per the standard method (Anonymous, 1987; 1998).

## TLC/HPTLC finger print analysis

### Preparation of extracts for TLC

Three batch samples of the drug (5g each) were extracted with 20 ml of chloroform and 20 ml of alcohol separately. Both the extracts were filtered and concentrated separately up to 10 ml in volumetric flask and were used for the TLC/HPTLC finger print analysis.

TLC/HPTLC finger print studies of chloroform and alcohol extracts of the drug were carried out using aluminum plate precoated with silica gel 60 $F_{254}$ (E. Merck) with CAMAG Linomat IV sample applicator. The chromatograms of both the extracts were taken using the solvent 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 various wavelengths. The plates were scanned at 254 nm to record the finger print spectrum after that same plates were visualized at UV-366 nm and derivatized with spraying of vanillin-sulphuric acid reagent and heated at 105° C till appeared coloured spots (Wagner and Bladt, 1984; Sethi, 1996).

### Estimation of microbial load

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

### Estimation of heavy metals

The method used for the analysis of heavy metals like lead, cadmium, mercury and arsenic was as per Guidelines & WHO (Anonymous, 1998) and AOAC (Anonymous, 2005).

### Details of the Instrument 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 vapor 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

Aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ were analyzed as per Official Analytical Methods of the American Spice Trade Association (ASTA), 1997.

### Details of instrument and operating parameters

High Performance Liquid Chromatography (Thermo Fisher) was used for the analysis of aflatoxins. 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 method used for the analysis of pesticide residues was as per AOAC (Anonymous, 2005). Pesticide 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 ìm).

## Results and Discussion

The prepared drug was obtained in brownish colour semi-solid, shows characteristic of its own odour and gives sweetish taste.

### Identification

### Microscopy

Parenchyma cells with wavy margin in surface view filled with dark reddish brown contents from the epidermis of the seed, perisperm cells in surface view filled with starch grains (Tukhm-e-Khurfa); elongated palisade like parenchyma and polygonal parenchyma cells from the cotyledons (Maghz-e-Tukhm-e-Petha); vessels with pitted thickening, transverse to oblique perforations with tail like projections at one or both ends (Sandal Safaid / Sandal Surkh); clusters of calcium oxalate crystals (Ood-e-Saleeb / Beikh-e-Anjabar) (upto 100µ in Beikh-e-Anjabar); prismatic crystals of calcium oxalate upto 500µ (Darunaj Aqrabi);

lignified elongated sclereids upto 260µ long (Mazu); lignified sclereids / stone cells of polygonal in shape upto 150µ (Ood-e-Saleeb) (Fig. 1).

## Physico-chemical parameters

Physico-chemical parameters of Majoon-e-Hamal Ambari Alwi Khani are tabulated in Table 2. Quantitatively evaluated data revealed that the moisture content was 19.68 %, ash content was 2.61 % and acid insoluble ash 1.44 %

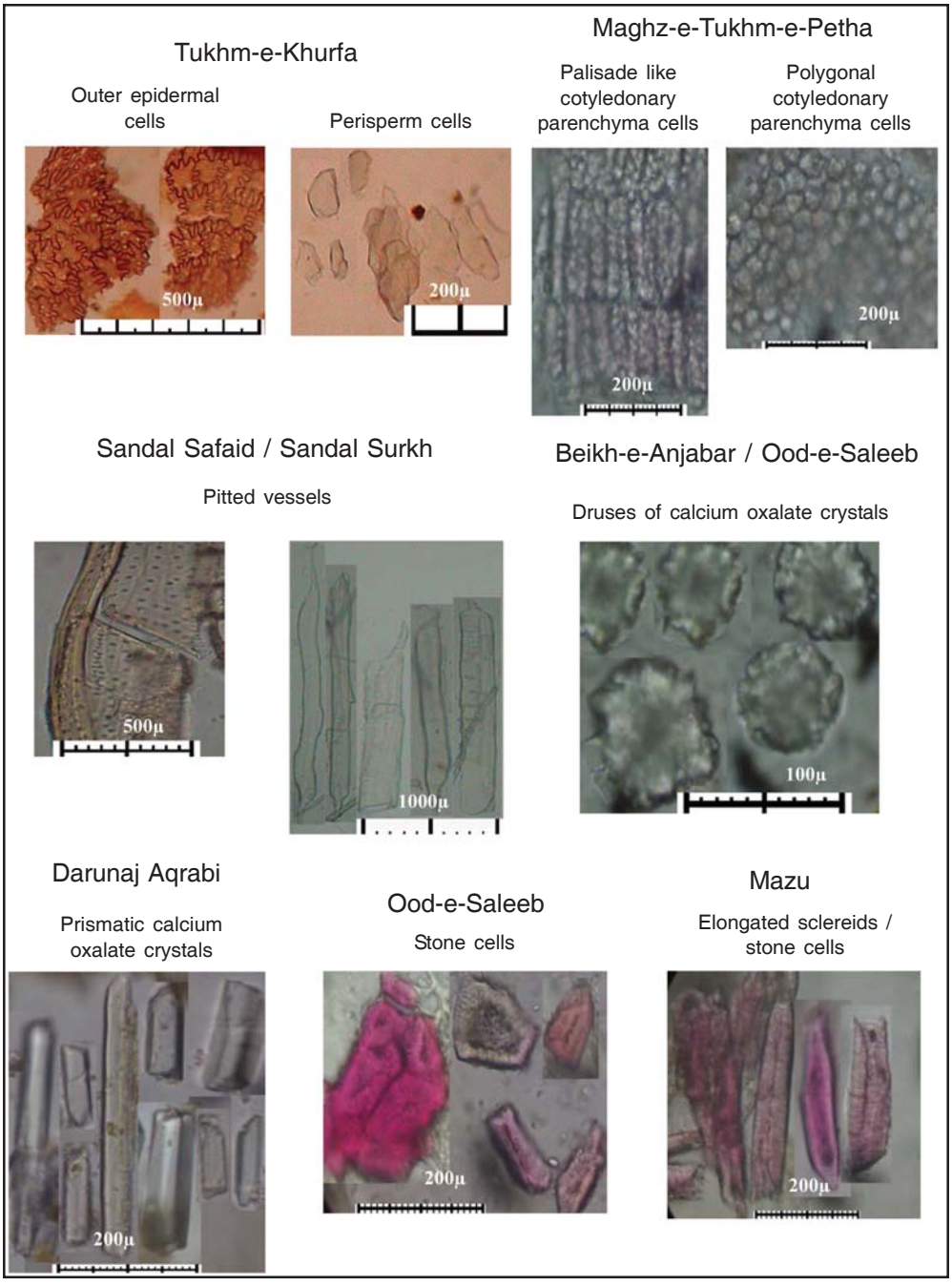



**Fig. 1:** Powder Microscopy

**Table 2:** Physico-chemical Parameters of studied drug

| S.No. | Parameters | Majoon-e-Hamal Ambari Alwi Khani | | |
|---|---|---|---|---|
| | | Batch - I | Batch - II | Batch - III |
| 1 | Moisture (% w/w) | 19.75 | 19.45 | 19.83 |
| 2 | Extractive values (% w/w) | | | |
| | Alcohol soluble matter | 26.14 | 26.53 | 26.74 |
| | Water soluble matter | 67.70 | 67.65 | 67.28 |
| 3 | Ash values (% w/w) | | | |
| | Total ash | 2.68 | 2.42 | 2.74 |
| | Acid insoluble ash | 1.45 | 1.34 | 1.53 |
| 4 | *pH* values | | | |
| | 1% Aqueous solution | 5.37 | 5.25 | 5.06 |
| | 10% Aqueous solution | 4.53 | 4.63 | 4.42 |
| 5 | Sugar estimation | | | |
| | Reducing sugar (% w/w) | 17.71 | 17.60 | 17.42 |
| | Non reducing sugar (% w/w) | 30.84 | 30.34 | 30.25 |
| 6 | Bulk Density | 1.4097 | 1.4207 | 1.4154 |
| All values are mean of three determinations | | | | |

indicated the negligible amount of siliceous matter present in the drug. The water soluble extractive value of the drug 67.54 % indicated the presence of inorganic and more polar organic content and the alcohol soluble extractive value 26.47 % indicated the extraction of polar constituents.

## TLC studies of chloroform extract

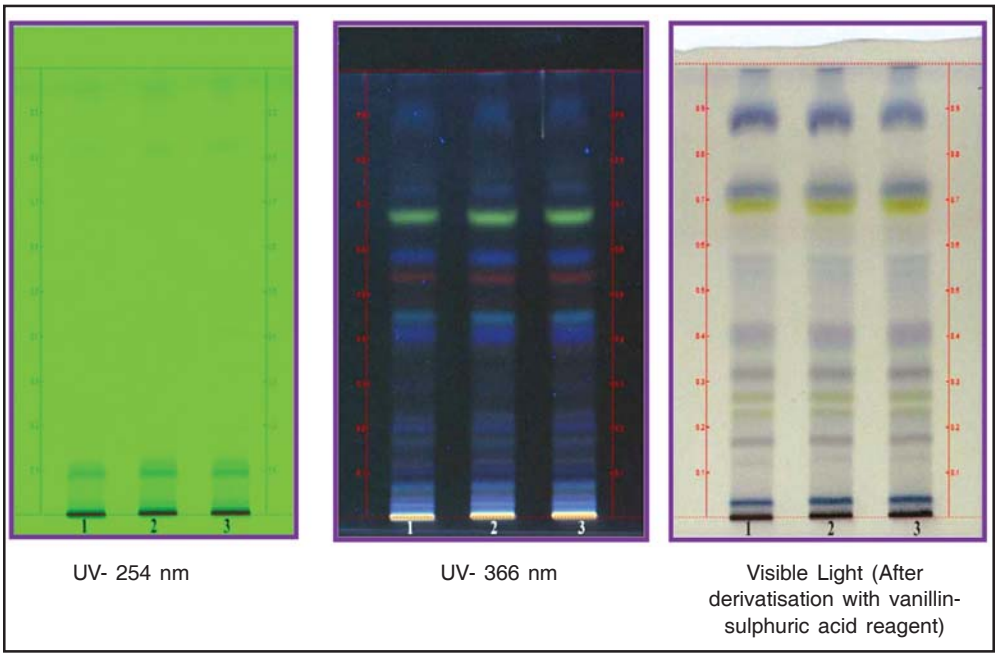
The TLC studies of chloroform extract are tabulated in Table 3. All the three batch samples showed identical spots in UV-254 nm, UV-366 nm and visible light (after derivatized with vanillin – sulphuric acid reagent). In UV – 254 nm, 366 nm and visible light it shows 06, 10 and 11 spots respectively with different $R_f$ values (Fig. 2).

## HPTLC finger print studies of chloroform extract

The finger print of the chloroform extract shows 14 peaks of which peaks at $R_f$ 0.11, 0.22 and 0.48 were the major peak whereas peaks at $R_f$ 0.03, 0.06, 0.27 0.37, 0.43, 0.60, 0.67, 0.77, 0.83, 0.89 and 0.99 were moderately smaller peaks (Fig. 3). The HPTLC densitometry chromatogram of chloroform extract of three batch samples of Majoon-e-Hamal Ambari Alwi Khani formulation were found to be same when scanned at 254 nm (Fig. 4)

**Table 3:** $R_f$ values of the chloroform extract of 'Majoon-e-Hamal Ambari Alwi Khani'

| Solvent System | *Rf* Values | | |
|---|---|---|---|
| | **UV- 254 nm** | **UV – 366 nm** | **Visible light (after derivatization with vanillin – sulphuric acid reagent)** |
| Toluene: Ethyl acetate (9:1) | 0.82 Green | 0.89 Blue | 0.90 Violet |
| | 0.73 Green | 0.73 Blue | 0.73 Violet |
| | 0.41 Green | 0.67 Yellowish green | 0.69 Yellowish green |
| | 0.31 Green | 0.59 Violet | 0.59 Gray |
| | 0.19 Green | 0.54 Brown | 0.54 Grey |
| | 0.10 Green | 0.45 Blue | 0.41Violet |
| | | 0.41 Violet | 0.32 Violet |
| | | 0.21 Blue | 0.26 Yellowish green |
| | | 0.17 Blue | 0.23 Yellowish green |
| | | 0.12 Blue | 0.16 Violet |
| | | | 0.12 Grey |




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

**Fig 2:** TLC photos of chloroform extract

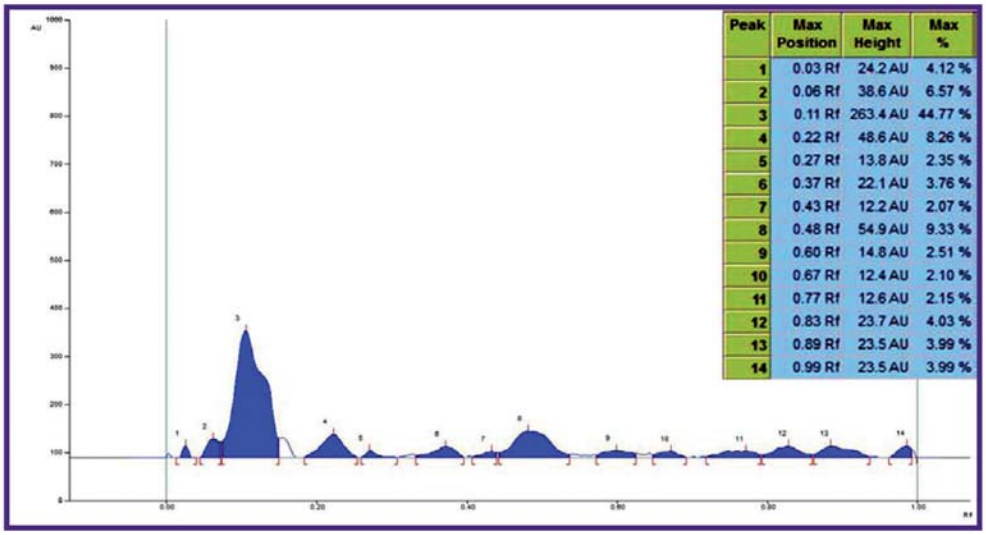



**Fig. 3:** HPTLC finger print profile of chloroform extract at 254 nm of Majoon-e-Hamal Ambari Alwi Khani formulation

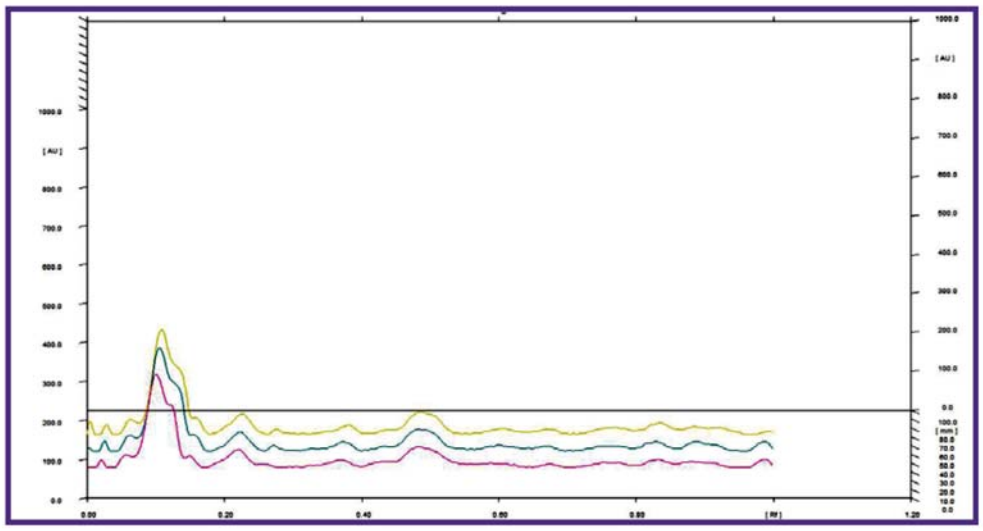


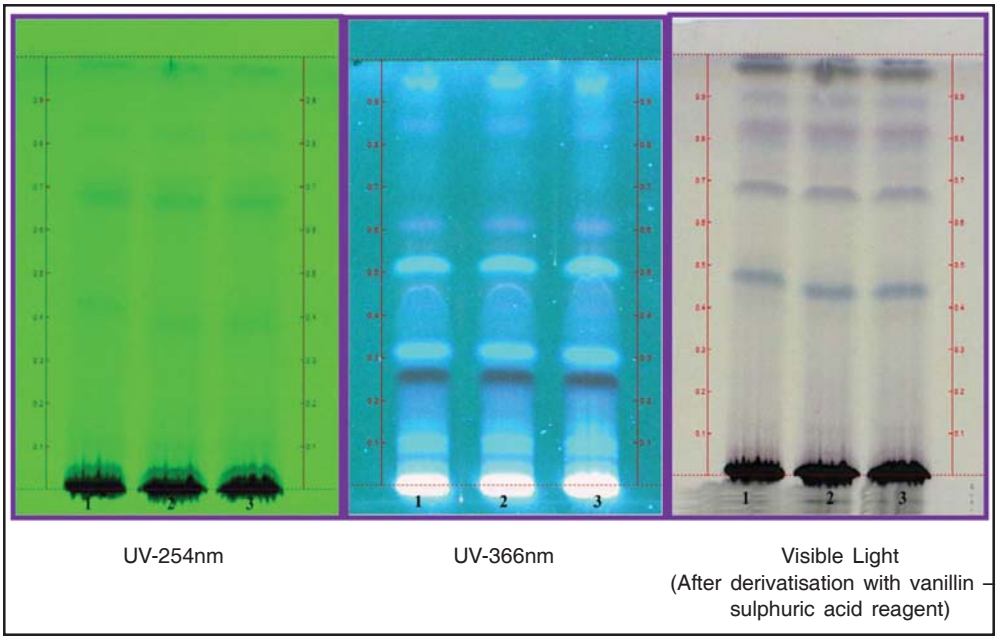
**Fig. 4:** HPTLC densitometry chromatogram of chloroform extracts of three batch of Majoon-e-Hamal Ambari Alwi Khani at 254 nm

## TLC studies of alcohol extract

The TLC studies of alcohol extract are tabulated in Table 4. All the three batches of Majoon-e-Hamal Ambari Alwi Khani formulation showed identical spot in UV-254 nm, UV-366 nm and visible light (after derivatized with vanillin – sulphuric acid reagent). In UV – 254 nm, 366 nm and visible light it shows 5, 10 and 6 spots respectively with different $R_f$ values (Fig. 5).

**Table 4:** $R_f$ values of the alcohol extract of 'Majoon-e-Hamal Ambari Alwi Khani'

| Solvent System | $R_f$ Values | | |
|---|---|---|---|
| | **UV- 254 nm** | **UV – 366 nm** | **Visible Light (After derivatisation with vanillin – sulphuric acid reagent)** |
| Toluene: | 0.83 Green | 0.89 Fluorescent blue | 0.90 Grey |
| Ethyl | 0.72 Green | 0.83 Fluorescent blue | 0.82 Red |
| acetate | 0.68 Green | 0.74 Blue | 0.69 Gray |
| (1:1) | 0.37 Green | 0.70 Blue | 0.44 Violet |
| | 0.10 Green | 0.66 Blue | 0.19 Grey |
| | | 0.57 Fluorescent blue | 0.11 Grey |
| | | 0.48 Fluorescent blue | |
| | | 0.41 Blue | |
| | | 0.21 Blue | |
| | | 0.12 Blue | |




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

**Fig. 5:** TLC photos of alcohol extract

HPTLC finger print studies of alcohol extract of 'Majoon-e-Hamal Ambari Alwi Khani' formulation

The finger print of the chloroform extract shows 10 peaks of which peaks at $R_f$ 0.02, 0.44, 0.77 and 0.94 were the major peak whereas peaks at $R_f$ 0.05, 0.11, 0.19, 0.47, 0.50 and 0.65 were moderately smaller peaks (Fig. 6). The HPTLC densitometry chromatogram of alcohol extract of three batch samples were found to be same when scanned at 254 nm (Fig. 7)

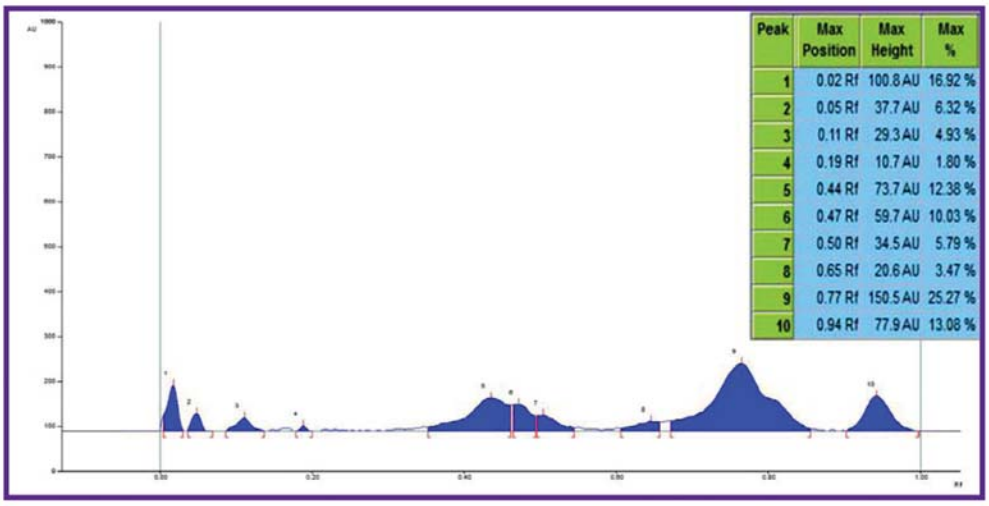



**Fig. 6:** HPTLC finger print profile of alcohol extract of Majoon-e-Hamal Ambari Alwi Khani formulation at 254 nm

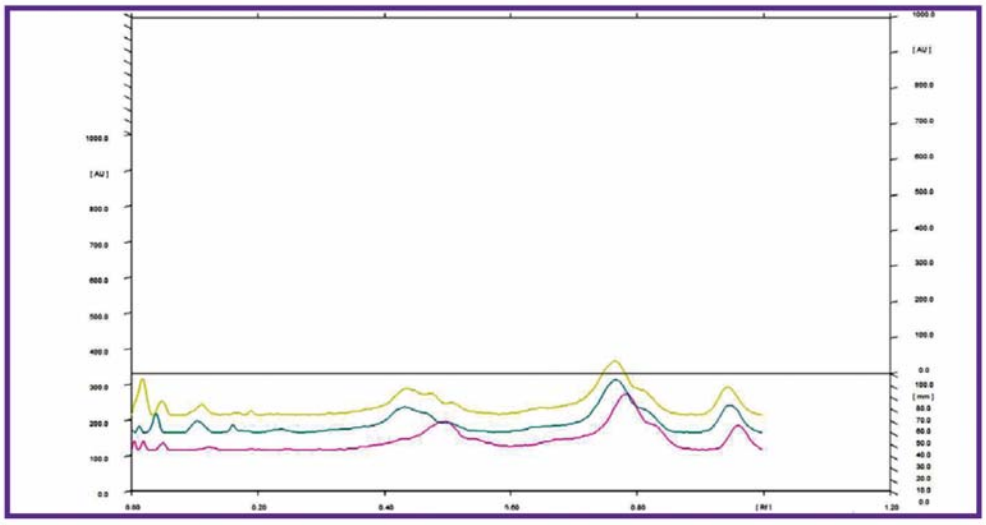


**Fig. 7:** HPTLC densitometry chromatogram of alcohol extracts of Majoon-e-Hamal Ambari Alwi Khani formulation at 254 nm

## Detection of quality parameters

In order to assess the quality of drug samples the estimation of microbial load viz. total bacterial count (TBC), total fungal count (TFC), Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* were analyzed and found to be in permissible limit. The results are shown in (Table 5). The heavy metal such as lead was present within the permissible limit where as cadmium; mercury and arsenic were not detected from the drug samples (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 reported in 'Majoon-e-Hamal Ambari Alwi Khani' formulation

| Parameters | Results | WHO Limits for internal use |
|---|---|---|
| Total Bacterial Count (TBC) | 4 x $10^3$ cfu/g | 1x$10^5$cfu/g |
| Total Fungal Count (TFC) | <10CFU 2 x $10^2$ cfu/g | 1x$10^3$cfu/g |
| Enterobacteriaceae | Absent | 1x$10^3$cfu/g |
| *Escherichia coli* | Absent | 1x$10^1$cfu/g |
| *Salmonella* spp | Absent | Absent |
| *Staphylococcus aureus* | Absent | Absent |

**Table 6:** Analysis of heavy metals in 'Majoon-e-Hamal Ambari Alwi Khani' formulation

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

## Conclusion

Standardization is an essential part for the evaluation of scientific standards to justify the quality of Unani herbal formulations. To maintain the batch-to-batch consistency and quality of the drug, each plant material used in preparation of 'Majoon-e-Hamal Ambari Alwi Khani' was identified and evaluated for their pharmacopoeial standards. The evaluated pharmacognostical and physico-

chemical parameters will be helpful for fixing-up pharmacopoeial standards of the drug. TLC/HPTLC finger print profile of chloroform and alcohol extracts provided a suitable method for monitoring the identity and purity and also standardization of the drug. Analyzed data of quality parameters viz. heavy metals, aflatoxins, pesticide residues and microbial load were found within permissible limit of WHO, which indicate that the studied drug 'Majoon-e-Hamal Ambari Khani' is free from toxic materials and can be used as uterine tonic, and also in the ailments of atony to the uterus and habitual abortion.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