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# STANDARDIZATION OF *MAJOON-E-AZARAQI* FORMULATION USED IN FACIAL PARALYSIS

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## ABSTRACT

In Unani System of Medicine, though the single drugs are used for many ailments, but compound formulations plays a major role in the treatment of various disorders. Evaluation of pharmacopoeial studies for herbal formulations is an essential part to assess the quality of drugs for therapeutic value. The World Health Organization (WHO) has given a detail protocol for the evaluation of herbal single drugs, but very little literature is available for the standardization of poly-herbal drugs. Majoon-e-Azaraqi is an ancient most commonly used Unani formulation prescribed for the ailments of Nerve strengthening, Hemiplegia, Luqwa, Facial paralysis, Tremor, Trembling, Rheumatism, Epilepsy and Neurasthenia. The poly herbal drug Majoon-e-Azaraqi was prepared in different batches with the combination of 15 ingredients. Three different batch samples prepared at laboratory scale were subjected to evaluate the physico-chemical, TLC/HPTLC finger prints, heavy metal, microbial load, aflatoxins and pesticide residues. The physico-chemical data such as moisture content was 19.52%, alcohol soluble extractives 32.66% and water soluble extractive 70.65% shows presence of polar compounds and inorganic materials respectively. The content of total ash was 1.44% and acid insoluble ash 0.34% shows negligible amount of siliceous matter present in the drug. TLC/HPTLC finger prints of chloroform and alcohol extracts were developed with the developing systems *toluene: ethyl acetate* - 9:1 and 6:4 respectively. All three different batch samples were found to be safe when tested for the heavy metal content, microbial load, aflatoxins and pesticide residues. This research work may be adopted for laying down the pharmacopoeial standards and TLC/HPTLC finger prints for Majoon-e-Azaraqi formulations.

**KEYWORDS**: *Majoon-e-Azaraqi*, Pharmacopoeial Standardization and HPTLC analysis.

## INTRODUCTION

To cure the various ailments, India has a rich heritage of traditional medicines like Ayurveda, Siddha and Unani. Herbal plants are the major part of these traditional medicines. Development of pharmacopoeial studies of these herbal medicines with the perspectives of safety, efficacy and quality will not only help to preserve the traditional heritage but also to rationalize the use of traditional medicines in the healthcare. The plant species mentioned in the ancient texts and other Indian systems of medicine may be explored with the modern scientific approaches for better leads in the healthcare.[1] Quality assurance of herbal medicine is an important factor and basic requirement for herbal drug industry and for other drug development organizations. Due to lack of standard operating procedure and quality control methods, there are batch to batch variations in the study of same formulation.[2]

The drug Majoon-e-Azaraqi is an ancient herbal Unani compound formulation which is therapeutically useful in the ailment of Nerve strengthening, Hemiplegia, Facial paralysis, Tremor, Trembling, Rheumatism, Epilepsy and Neurasthenia.[3] The present study was an attempt to evaluate the pharmacopoeial studies such as organoleptic characters, microscopic, physico-chemical, HPTLC and quality control parameters.

## MATERIAL AND METHODS

### Ingredients authentications

The raw drugs namely Azaraqi Mudabbar, Berg-e-Gaozaban, Ustukhuddus, Kateera, Narjeel, Maghz-e-Chilghoza, Dana Heel Khurd, Zarambad, Shaqaq-ul-Misri, Sandal Safaid, Aamla, Halela Siyah, Ood Hindi, Qaranfal and Qand Safaid were procured from local market, Chennai and were authenticated as per pharmacopoeial and other official standards.[4-9]

### Drug preparation

### Formulation Composition[3]

| | | | | |
|---|---|---|---|---|
| 1. | Azaraqi Mudabbar UPI-II | *Strychnos nuxvomica* Linn. | Seed | 30g |
| 2. | Berg-e-Gaozaban UPI-II | *Borago officinalis* Linn. | Leaf | 20g |
| 3. | Ustukhuddus | *Lavandula stoechas* Linn. | Flower | 15g |
| 4. | Kateera UPI-VI | *Cochlospermum religiosum* Linn. | Gum | 15g |
| 5. | Narjeel API-III | *Cocos nucifera* Linn. | Endosperm | 15g |
| 6. | Maghz-e-Chilghoza UPI-VI | *Pinus gerardiana* Wall. | Kernel | 15g |
| 7. | Dana Heel Khurd UPI-I | *Eletarria cardamomum* (L.) Maton | Seed | 10g |
| 8. | Zarambad API-IV | *Curcuma zeodaria* Linn. | Rhizome | 10g |

| | | | | |
|---|---|---|---|---|
| 9. | Shaqaq-ul-Misri UPI-III | *Pastinaca secacul* Linn. | Rhizome | 10g |
| 10. | Sandal Safaid UPI-VI | *Santalum album* Linn. | Heartwood | 10g |
| 11. | Aamla UPI-I | *Emblica officinalis* Gaertn. | Fruit | 10g |
| 12. | Halela Siyah UPI-I | *Terminalia chebula* Retz. | Fruit | 10g |
| 13. | Ood Hindi UPI-VI | *Aquilaria agallocha* Roxb. | Heartwood | 5g |
| 14. | Qaranfal UPI-I | *Syzygium aromaticum* (L.) Merr. L M Perry | Flower bud | 5g |
| 15. | Qand Safaid | Sugar | -- | 675g |

UPI=UNANAI PHARMACOPOEIA OF INDIA.

**Method of Preparation**

All the ingredients were taken of pharmacopoeial quality. The ingredient number 1 to 4 and 7 to 14 were cleaned, dried, powdered and sieve through 80 mesh and kept separately. Ingredient number 5 and 6 were coarsely powdered and sieve through 60 mesh. The ingredient number 15 was dissolved in 750 ml of water on slow heat; at the boiling stage 0.1% citric acid was added and mixed thoroughly. The content was boiled and added the 0.1% sodium benzoate at 72% consistency of quiwam and mixed well. Then quiwam was re-corrected of 77% consistency. Then the vessel was removed from the fire, while hot condition mixed powder of ingredient number 1 to 14 was added and mixed thoroughly to prepare the homogenous product. Finally, it was cooled to room temperature and packed in tightly closed containers to protect from light and moisture.

**Pharmacopoeial standards**

**Organoleptic Evaluation**

Organoleptic evaluation refers to assess the formulation by smell, colour, odour and taste and were carried out based on the method described by Siddique *et. al.*, (1995).[10]

**Powder microscopical studies**

The drug sample (5g) was weighed and mixed with 50 ml 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 was taken in watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid and mounted in glycerine. The salient features of the drug were observed in different mounts.[11]

**Physico-chemical analysis**

The moisture content at 105°C, ash values, solubility in water and alcohol, *pH* values, bulk density and sugar content are the useful parameters in standardisation of herbal products were analyzed as per standards method.[12-13]

**TLC/HPTLC finger print analysis**

**Preparation of extracts for TLC**

The prepared three different batch samples were extracted with chloroform and alcohol. The extracts were concentrated and made up to 10 ml in a volumetric flask separately. These extract solutions were used for the TLC/HPTLC finger print analysis.

TLC/HPTLC finger prints of chloroform and alcohol extracts of the formulations were performed using Aluminum plate precoated with silica gel 60 $F_{254}$ (E.merck) employing CAMAG Linomat-IV sample applicator. The chromatogram were developed 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 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 reagent* and heated at 105° C till appeared the colored spots.[14]

**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[13], 1998.

**Estimation of Heavy Metals**

The procedure used for the analysis of heavy metals like lead, cadmium, mercury and arsenic was as per WHO[13], 1998 and AOAC[15], 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 vapor 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**

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).[16]

**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 - 35ºC; detector - fluorescence detector at 360 nm; injection - 20µl (Aflatoxins mixture and sample).

**Analysis of pesticide residue**

The procedure followed for the analysis of pesticide residues was as per AOAC[16], 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-0.25µm).

## RESULTS AND DISCUSSION

**Organoleptic characters**

The drug Majoon-e-Azaraqi is brown in colour, semi-solid, has characteristic of its own odour and bitter in taste.

**Microscopical observations**

Thick walled epidermal cell with abundant fragments of lignified rods of trichomes: endosperm thick walled cellulosic parenchyma containing fixed oil and aleurone grains **(Azaraqi);** epidermal cells with anomocytic, anisocytic stomata and glandular trichomes,

unicellular covering trichomes **(Berg-e-Gaozaban**); pollen grains small, spherical to ellipsoidal upto 45µ with six furrows, six germ pores (hexacolpate), line of pits radiating from the pores, branched or tufted multicellular trichomes and glandular trichomes **(Ustukhuddus);** elongated thin walled parenchyma cells from the mesocarp filled with oil globules **(Narjeel)**; cotyledonary parenchyma cells **(Maghz-e-Chilghoza);** perisperm cells isolated or in groups with bulbous projections filled with starch grains and tiny prismatic crystal of calcium oxalate, elongated cells of thin walled parenchyma cells from aril tissue, thick walled sclerenchyma cells filled with reddish brown contents **(Dana Heel Khurd);** numerous starch grains of various shapes and size upto 50µ **(Zarambad);** long lignified vessels with reticulate and scalariform thickenings of upto 45µ breadth, raphides of calcium oxalate crystals upto 100µ **(Shaqaq-ul-Misri);** pitted vessels of length upto 1500µ and breadth upto 70µ with transverse to oblique perforations with tail like projections at one or both the ends, pitted vessels isolated or associated with fibres **(Sandal safaid);** epidermal cells with uniformly thick walled cells containing silica crystals and occasional paracytic stomata, mesocarpic parenchyma cells with large irregular thick walled cells showing corner thickening **(Aamla)**; epidermal cells with uniformly thick walled cells, several of them divided into two by a thin septa, groups of thick walled stone cells or sclereids with pitted walls and broad lumen, fragments of crisscross fibres and fibres with peg like outgrowths and simple pitted walls **(Halela Siyah);** pitted vessels upto 175µ filled with dark brown contents **(Ood Hindi)**; pollen grains tetrahedral spherical biconvex measuring upto 20µ, sclerenchymatous spindle shaped pericyclic fibres of breadth upto 40µ, parenchyma cells with schizolysigenous oil glands, fragments of anther wall in surface view **(Qaranfal) (Fig.1).**

**Physico-chemical**

The physico-chemical analysis such as moisture content obtained in the drug was 19.52%. The alcohol soluble extractive (32.66%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (70.65%) indicate the presence of inorganic constituents. The obtained data are shown in (**Table – 2)**.

**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 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 **(Fig 2 and 5).**

**Quality control parameters**

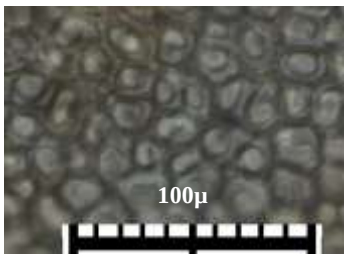
The evaluated quality control parameters such as microbial load and heavy metals were found within the permissible limit in the drug shown in **(Table - 5 and 6)**. The other parameters like aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ and pesticide residues were not detected from the drug samples shown in (**Table - 7 and 8)**.

**CONCLUSION**

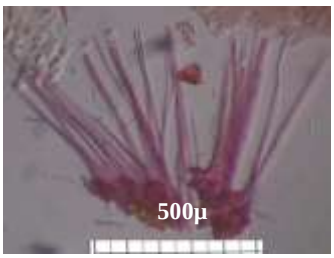
In the present study the Unani formulation has been standardized by modern scientific quality control measures. The results obtained from these pharmacopoeial parameters could be used to analyse the formulations and to check the quality and batch-to-batch variations.

**Azaraqi**

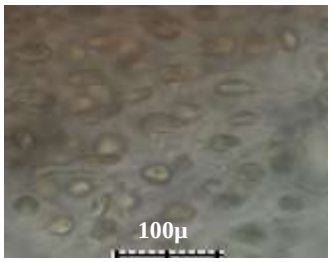
Epidermal cells




Trichomes thick walled and lignified

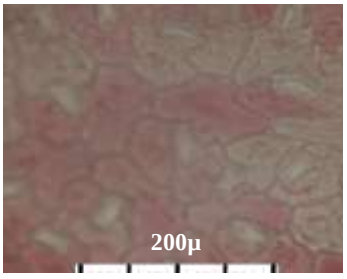



Endosperm cells

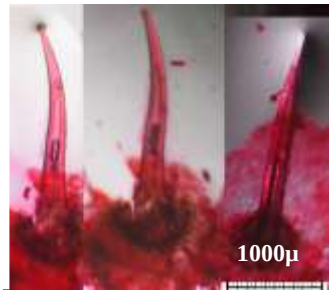



**Berg-e-Gaozaban**

Epidermal cells with anomocytic and anisocytic stomata




Unicellular covering trichomes

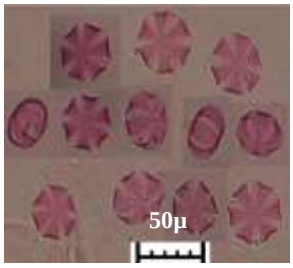



**Maghz-e-Chilghoza**

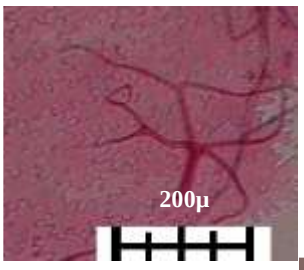
Cotyledonary parenchyma cells

## Ustukhuddus

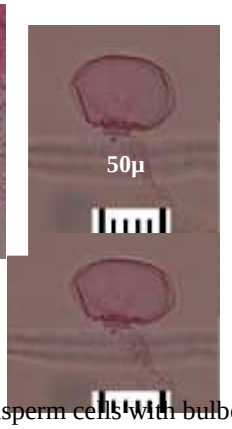
Pollen grains




Trichome

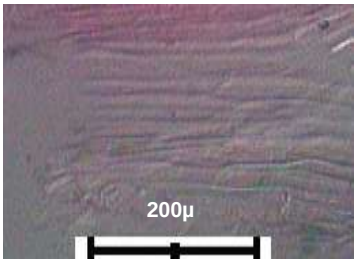



Glandular trichome



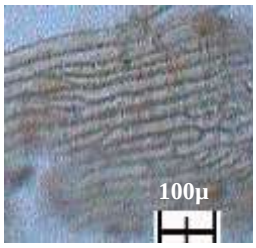

## Narjeel

Elongated parenchyma cells with oil globules

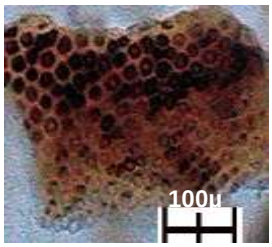



## Dana Heel Khurd

Aril tissue



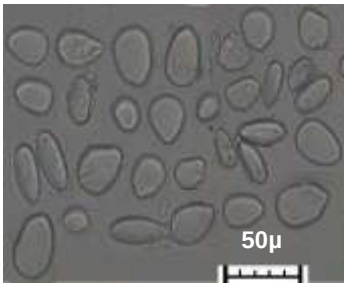

Thick walled sclerenchyma cells




Perisperm cells with bulbous projections packed with starch grains

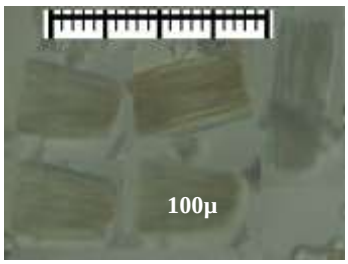

## Zarambaad

Starch grains




## Shaqaq-ul-Misri

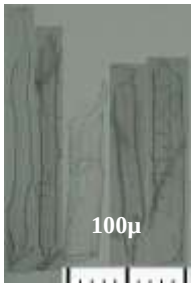
Raphides



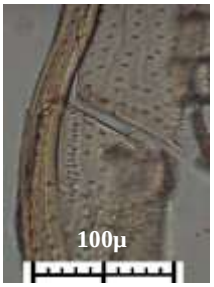

Reticulate and Scalariform vessels


## Sandal Safaid

Pitted vessels

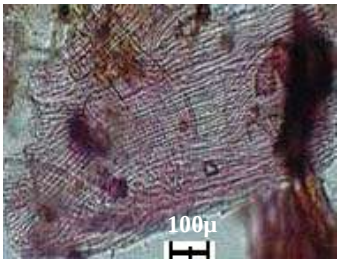



Fibre associated with vessel

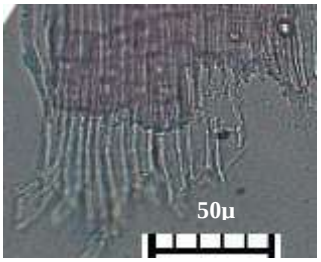



## Halela Siyah

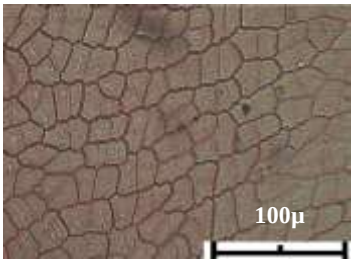
Criss cross fibres




Criss cross fibres with pegged tips




Epidermal cells in surface view



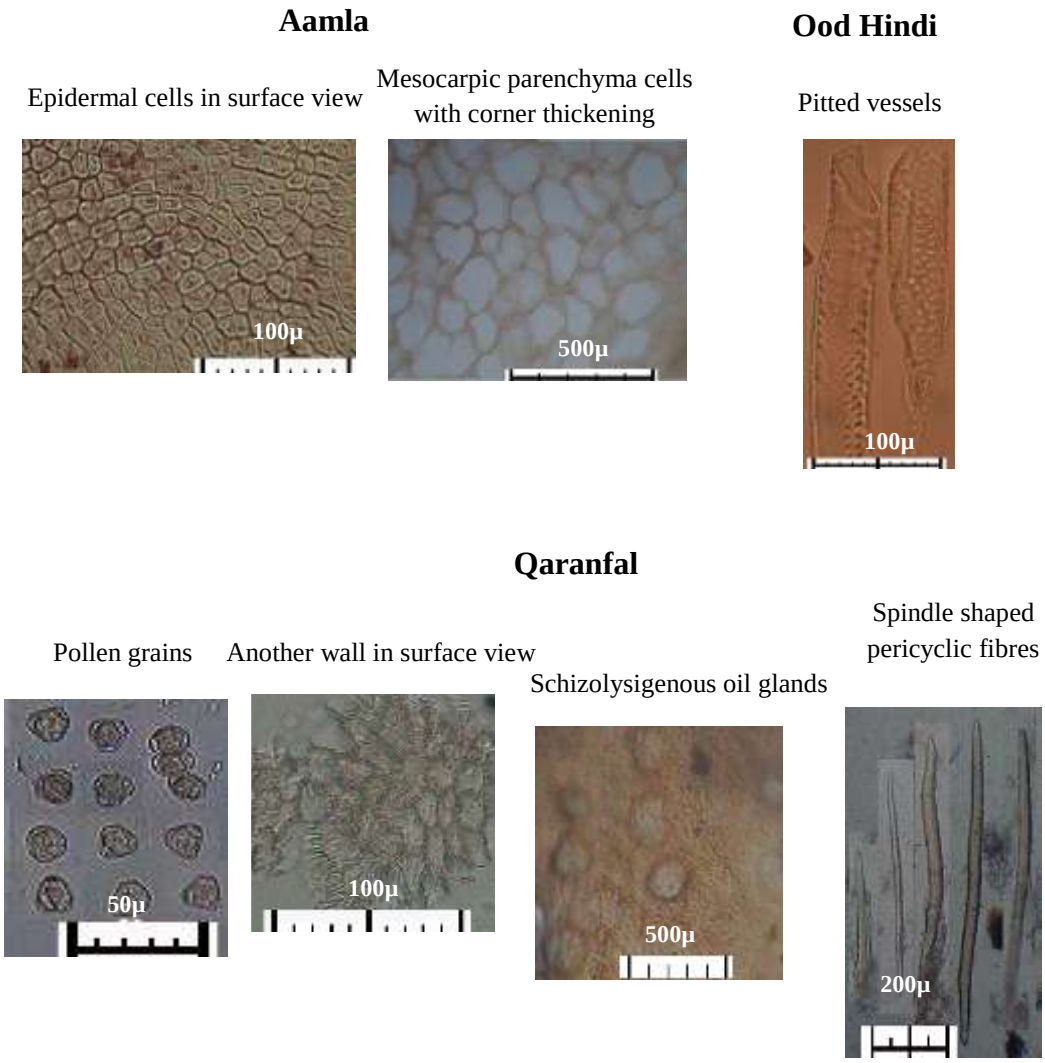



**Fig.1 MAJOON-E-AZARAQI.**

**Table – 2. Physico-Chemical parameters.**

| Parameters Analyzed | Batch Number (n=3) | | |
|---|---|---|---|
| | I | II | III |
| **Extractives**<br>Alcohol soluble matter<br>Water soluble matter | 32.56%<br>70.52% | 32.80%<br>70.61% | 32.63%<br>70.84% |
| **Ash**<br>Total ash<br>Acid insoluble ash | 1.35%<br>0.41% | 1.49%<br>0.37% | 1.50%<br>0.33% |
| **pH values**<br>1% Aqueous solution<br>10% Aqueous solution | 5.36<br>4.53 | 5.52<br>4.79 | 5.21<br>4.49 |
| **Sugar estimation**<br>Reducing sugar<br>Non-reducing sugar | 31.18%<br>10.14% | 31.32%<br>10.20% | 31.51%<br>10.42% |
| **Moisture** | 19.41% | 19.74% | 19.84% |
| **Bulk Density** | 1.5399 | 1.5308 | 1.5109 |

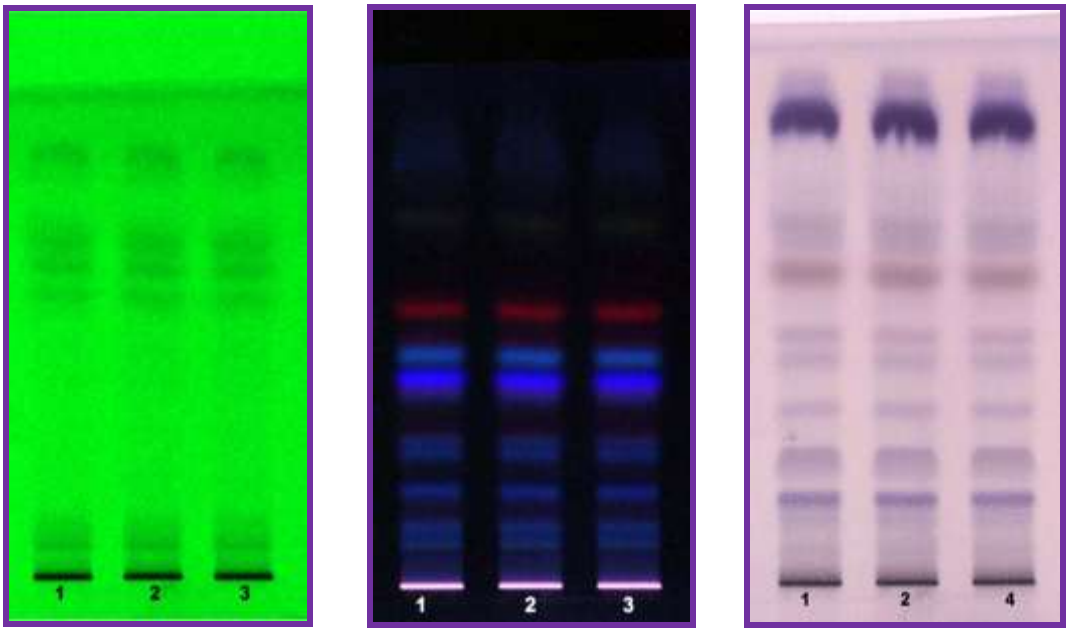
**Thin Layer Chromatography of Chloroform Extract**

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 4, 9 and 8 spots respectively with different $R_f$ values **(Fig 2).**

**Table – 3, Rf values of the Chloroform Extract.**

| Solvent System | *Rf* Values | | |
|---|---|---|---|
| | **UV- 254 nm (4 Spots)`** | **UV – 366 nm (9 Spots)** | **Visible light (After derivatization with vanillin – sulphuric acid reagent) (8 Spots)** |
| **Toluene: Ethyl acetate (9:1)** | 0.87 Green | 0.88 Light blue | 0.90 Violet |
| | 0.67 Green | 0.55 Red | 0.68 Grey |
| | 0.62 Green | 0.50 Red | 0.59 Brown |
| | 0.56 Green | 0.46 Fluorescent blue | 0.48 Grey |
| | | 0.41 Fluorescent blue | 0.43 Grey |
| | | 0.38 Pink | 0.34 Grey |
| | | 0.28 Blue | 0.24 Grey |
| | | 0.19 Blue | 0.16 Violet |
| | | 0.11 Blue | |

**THIN LAYER CHROMATOGRAPHY (Chloroform Extract)**




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

**Fig-2.**

**HPTLC finger print studies of chloroform extract**

The finger print of the chloroform extract shows 8 peaks of which peaks at $R_f$ 0.07, 0.63, 0.70 and 0.75 were the major peak whereas peaks at $R_f$ 0.11, 0.22, 0.51 and 0.95 were moderately

smaller peaks **(Fig.3).** The HPTLC densitometry chromatogram of chloroform extract of three batch samples were found to be same when scanned at 254 nm **(Fig. 4)**

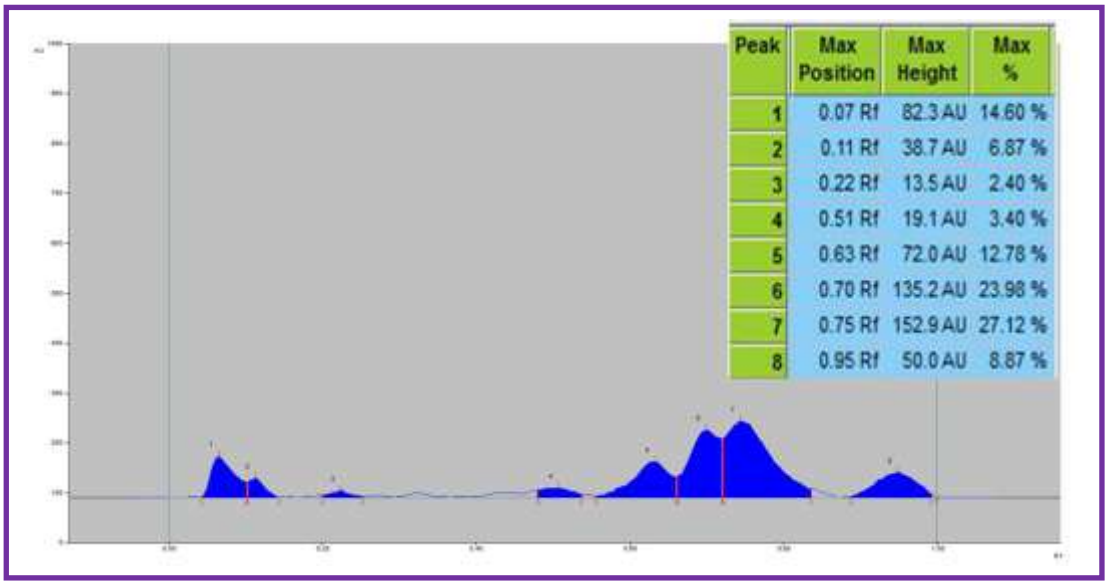



**Fig.-3 HPTLC Finger print of Majoon-e-Azaraqi of Chloroform extract at 254 nm.**

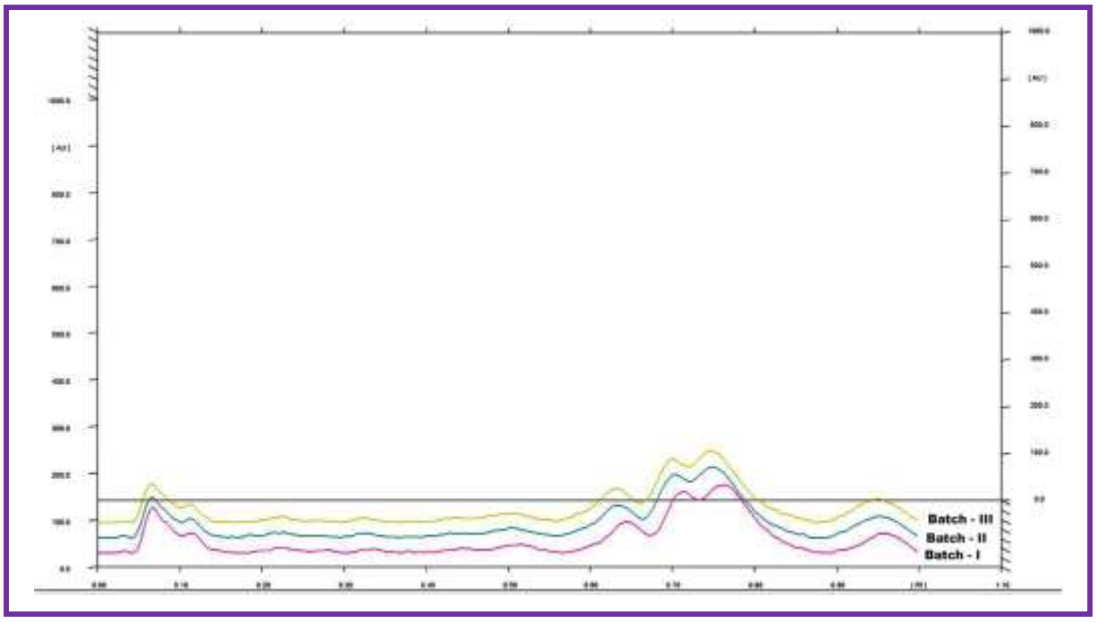



**Fig. -4 Densitometric Chromatogram of Majoon-e-Azaraqi of Chloroform extracts at 254nm.**

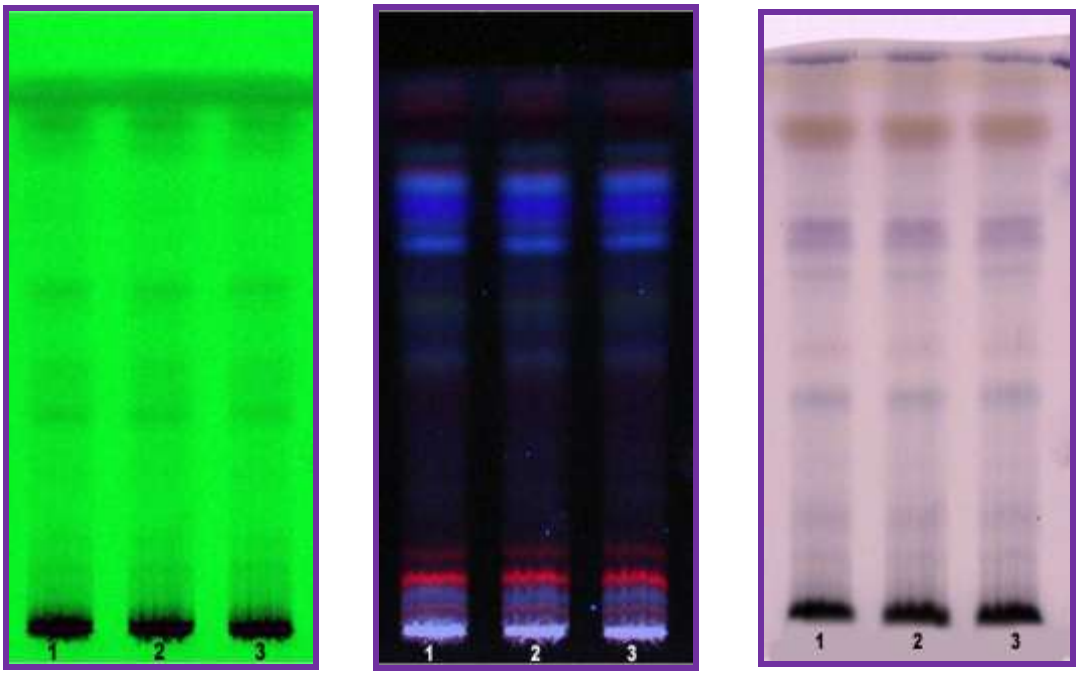
**TLC studies of Alcohol Extract**

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 derivatised with vanillin – sulphuric acid reagent). In UV – 254 nm, 366 nm and visible light it shows 6, 10 and 8 spots respectively with different $R_f$ values (**Fig. 5).**

**Table-4. $R_f$ Values of the Alcohol Extract.**

| Solvent System | $R_f$ Values | | |
|---|---|---|---|
| | UV- 254 nm (7 Spots) | UV – 366 nm (10 Spots) | Visible Light (After derivatisation with vanillin – sulphuric acid reagent) (8 Spots) |
| Toluene: Ethyl acetate (6:4) | 0.91 Green | 0.95 Red | 0.89 Brown |
| | 0.86 Green | 0.83 Blue | 0.70 Violet |
| | 0.60 Green | 0.82 Red | 0.62 Grey |
| | 0.47 Green | 0.80 Fluorescent blue | 0.48 Light grey |
| | 0.39 Green | 0.76 Violet | 0.39 Grey |
| | 0.16 Green | 0.69 Fluorescent blue | 0.27 Light grey |
| | 0.11 Green | 0.59 Yellowish green | 0.18 Grey |
| | | 0.50 Blue | 0.11 Grey |
| | | 0.15 Red | |
| | | 0.11 Red | |

**THIN LAYER CHROMATOGRAPHY (Alcohol Extract)**




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

**Fig.- 5.**

**HPTLC finger print studies of alcohol extract**

The finger print of the chloroform extract shows 9 peaks of which peaks at $R_f$ 0, 0.42, 0..52, 0.68 and 0.96 were the major peak whereas peaks at $R_f$ 0.01, 0.11, 0.18, 0.31 and 0.84 were moderately smaller peaks **(Fig. 6).** The HPTLC densitometry chromatograms of alcohol extract of three batch samples were found to be same when scanned at 254 nm **(Fig. 7).**

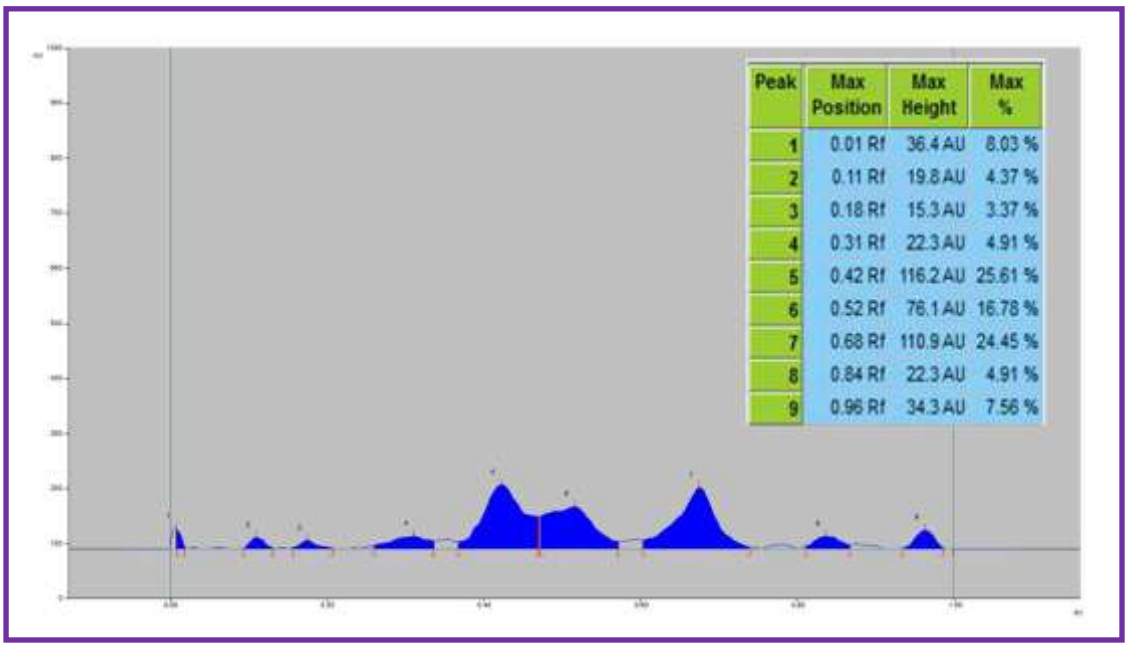



**Fig.- 6 HPTLC finger print of Majoon-e-Azaraqi alcohol extract at 254 nm.**

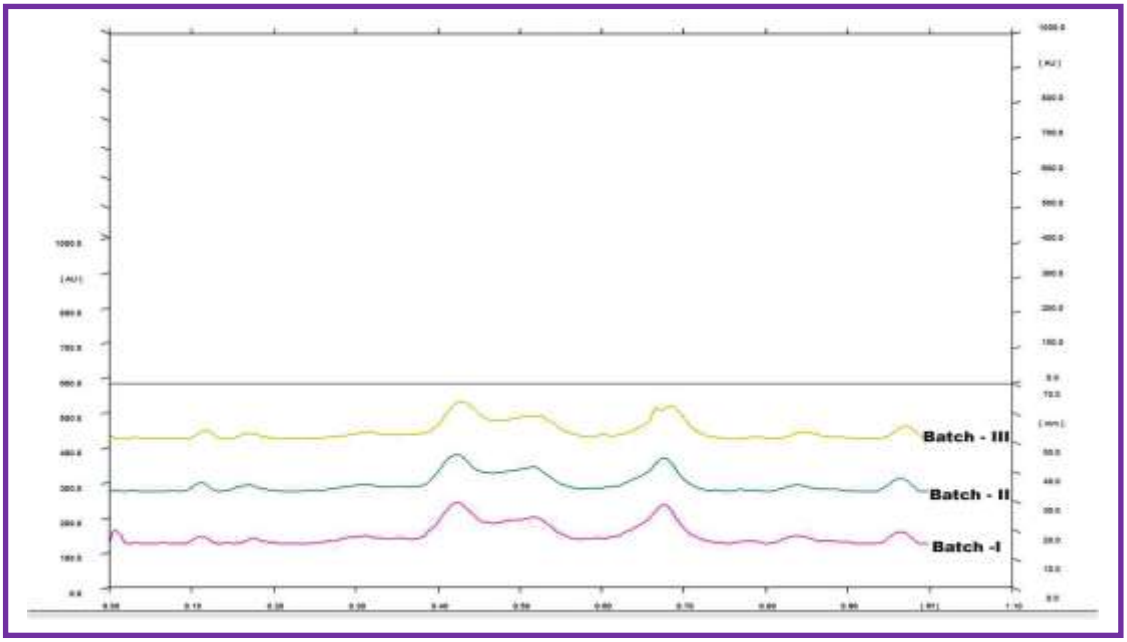



**Fig.-7 Densitometric chromatogram of Majoon-e-Azaraqi alcohol extracts at 254 nm.**

**Table - 5: Analysis of Microbial load.**

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

**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 | 0.0073ppm | 10 ppm |
| 4 | Mercury | Not detected | 1.0 ppm |

**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 |

**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 |

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