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Scientific Standard Validation and HPTLC. Finger printing studies of Polyherbal Unani Formulation Majoon-e-Maddat-ul-Hayat Jadwari

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ABSTRACT

The polyherbal drug Majoon-e-Maddat-ul-Hayat Jadwari has been used as a traditional and alternative medicine to treat and cure therapeutically in the treatment of Nisyan (Amnesia), Salasul Baul (Urinary Incontinence), Waja-ul-Kulya (Renal pain /Nephralgia), Waj-ul-Qutn (Lumbago), Waj-ul-Mafasil (Rheumatoid / Poly Arthritis) and has also been used Action wise Muqawwi-e-Asab, (Nervine Tonic), Muwallid-i-Mani (Spermatogenic), Hazim (Digestive), Daf-e-waja (Analgesic), Muqawwi-i-Bah (Aphrodisiac). In the present research study is aimed to evaluate the product drug standardization and quality validation using physico-chemical parameters; HPTLC fingerprinting, essential WHO and AYUSH Pharmacopeial parameters as per WHO and AYUSH guidelines protocols of analyzed parameters. Drug Standardization and product validation is very essential tools for validation and measured the batch to batch variation or uniformity of ASU herbal product. Therefore we need to develop standard validation techniques to standardize and validate of the poly herbal formulation. The drug MEMUHJ was prepared in three different batches as per the guidelines of Classical text / literatures of NFUM Vol, IVth Edition Ist and IInd. The physico-chemical average reading data's of every III Batches of test samples showed that the drug contain LOD/ Moisture ,w/w- (6.42%,6.69%,6.56%), Total ash, w/w- (1.66%,1.78%,1.66%), Acid in-soluble ash, w/w-(0.266%,0.323%,0.318%), Alcohol (ASEM) and Water (WSEM) soluble extractive matter, w/v- (53.66%,54.66%,55.04%) & (73.48%,74.01%, 74.41%) and Alcohol soluble successive extractive value (ASSEV). w/v- 54.92%,54.96%,54.90% , Chloroform soluble successive extractive value (CSSEV). w/v- 16.85%,16.72%,16.82%, pH (1% solution) (4.58,4.55, 4.51),pH(10% solution) (5.45,5.57, 5.75), Bulk density ,gm/ml - (1.525,1.515, 1.518),Reducing Sugar (54.31, 55.40, 54.80) and Non-Reducing Sugar

(23.43,23.52,23.50) the HPTLC finger prints showed various spots at 254nm, 366nm and visible light (V-S reagent). The validation of quality control study revealed the absence of Microbial load, Aflatoxins, Heavy metal and Pesticide residues, The evaluated validated quality standards will be very useful for referential support, pharmaco-vigilance and validation of the standards of MEMUHJ providing the quality medicine to needful human being.

Keywords: Majoon-e-Maddat-ul-Hayat Jadwari, Physico-chemical quality, Quality Control and Assurance parameters, TLC/ HPTLC fingerprinting and Unani Compound drug.

I. INTRODUCTION

It is also observed that about 80% of the world population is using medicinal plants primarily in the developing countries for treating different diseases, due to their safety, efficacy, cultural acceptability and lesser side effect. It is important for herbal formulations to get the quality assurance by the conventional system of medicine, so that they can be justified, accepted and must be beneficial for the ailing masses of the mankind (Madhav NVS *et al*., 2011; Naaz A *et al*., 2021). The quality assurance and quality control of herbal crude drugs and formulated products are important in justifying their acceptability in modern system of medicine. Hence it is required to conduct the research on drugs standardization and product validation to provide effective, curable and safe drugs to the needy mass suffering from various ailments.(Sagar *et al*.,2020) In the past decades there is a sense of awareness among the developing world population , about the importance of traditional system of medicines such as Unani, Ayurveda and Siddha in maintaining health without the side or adverse effects. Due to this scientific awareness a scenario has created to research activities like quality standardization and drug validation of traditional medicines and development of scientific methods for the manufacture of quality medicines.(Ramasamy *et al*.,2009). According to the WHO, the quantity, quality, safety and efficacy data on traditional medicine (TM) are not sufficient to meet the criteria needed, so some of the major policy challenges include safety, efficacy, quality, and enlightened the perception for the use of TM. Various policy measures have been applied for a cleareyed view of the use of TM, in order to increase its safety, efficacy and acceptability (G. Bodeker and G. Burford, 2007). As there is increase demand of herbs and herbal products especially Unani medicinal products, run across many problems like non-availability of good quality of raw materials, proper methodology for standardization. In consequence to ensure and develop the quality, authenticity of Unani formulations, the standardization of single as well as compound drugs on modern analytical parameter is basic requirement for drugs. Before studying pharmacological activity of any drug physico-chemical characteristics is necessary for its authenticity (Alam A *et al*., 2019; Naaz A *et al*., 2021).

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). Historically, herbal medicines have played a significant role in the management of both minor and major medical illness

(Bahuguna *et al.*,2014). All medicines, either synthetic or plant origin, have to fulfil the basic requirements of safety and efficacy. (EMEA, 2005; Anonymous, 2002)

The Standardization and Validation of ASU herbal Drugs is not an easy challenge as various factors influence the bio efficacy and reproducible therapeutic effects. In order to obtain assured quality based herbal products, care through pharmaco-vigilance and care should be taken right from the proper identification of plants, season and area of collection, grading, drying, extraction, purification process and rationalizing the combination in the case of poly-herbal drugs. (Patel *et al.*,2006), The subject of standardization of herbal drugs is massively wide and deep. There are many seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. (Yadav *et al.*,2011), For the purpose of drug standardization research work of herbal formulated products, a complete profound knowledge is of utmost important. 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)

The drug Majoon-e-Maddat-ul-Hayat Jadwari is one the classical Unani poly-herbal compound formulation. It is therapeutically useful in the ailment like Nisyan (Amnesia), Salasul Baul (Urinary Incontinence), Waja-ul-Kulya (Renal pain / Nephralgia), Waj-ul-Qutn (Lumbago), Waj-ul-Mafasil (Rheumatoid / Poly Arthritis). The Unani drug standardization research studies of the drug Majoon-e-Maddat-ul-Hayat Jadwari is frequently recommended as agent for palsy, piles and intestinal pain. In most of the Asian, European and Arabian countries it is used since ancient times as traditional and alternative medicine. The drug has been used and Action wise reported has also been used Action wise Muqawwi-e-Asab, (Nervine Tonic), Muwallid-i-Mani (Spermatogenic), Hazim (Digestive), Daf-e-waja (Analgesic), Muqawwi-i-Bah (Aphrodisiac). Majoon-e-Maddat-ul-Hayat Jadwari is a dark brown semi-solid preparation with characteristic of its own smell form preparation with agreeable, aromatic odour and sweet taste. Majoon-e-Maddat-ul-Hayat Jadwari was reported bioactive to contain active phytochemical constituents such as Alkaloids, Glycosides, Terpenoids, Tannins, Crude fibres, Sucrose, Fructose etc. The preparation of the drug in different batches is based on traditional methods in accordance with the procedure given in classical text and authenticated literatures mentioned in Hkm. Mohammad Sharif Khan.1921(A,D,); Hkm. Mohammad Hadi Hussain Khan Muradabadi.2005; NFUM, Part-IV, Ist Edition (Anonymous, 2001) and NFUM. Part-IV, IInd Edition (Anonymous, 2022).

II. METHODS AND MATERIAL

Ingredients used for preparation: The raw drug formulation is composed of the following mention ingredients:

S. No.	Unani Name	Botanical/ English Name	Part used	Reference	Qty.
1.	Filfil siyah	*Piper nigrum* L.	Fruit	UPI, Part I, Vol. IV, P. 38	15 g

2.	Filfil Daraz	*Pier longum* L.	Fruit	API, Part I, Vol. IV, p.105	15 g
3.	Dalchini	*Cinnamomum zeylanicum* Blume	Stem bark	UPI, Part I,Vol. I, p. 26	15 g
4.	Jadwar Khatai	*Delphinium denudatum* Wall.	Root	UPI, Part I , Vol. VI, p.31	25 g
5.	Ood Saleeb	*Paeonia officinalis*	Root tubers	UPI, Part I, Vol. III, p.74	25 g
6.	Bahman Surkh	*Saliva haematodes* M.	Root	UPI, Part I, Vol. III, p.23	25 g
7.	Aamla	*Emblica officinalis* Gaertn.	Fruit	UPI, Part I, Vol. I, p.5	30 g
8.	Marwareed Nasufta	*Mytilus margaritiferus*	Pearl(powder)	---	30 g
9.	Post-e-Balela	*Terminalia bellerica* Roxb.	Fruit pericarp	UPI, Part I, Vol. I, p. 17	50 g
10.	Sheetraj Hindi	*Plumbago zeylancia* L.	Root	UPI, Part I, Vol. I, p.80	50 g
11.	Zarawand	*Aristolochia rotunda* L.	Root	UPI, Part I, Vol. V, p. 109	50 g
12.	Gul-e-Babuna	*Matricaria chamomilla* L.	Flower	UPI, Part I, Vol. II, p. 39	50 g
13.	Khusyat-us-Salab	*Orchis latifolia* L.	Root tubers	UPI, Part I, Vol. III, p.88	50 g
14.	Nabat Safaid	*Cane sugar*	Crystal	---	50 g
15.	Chilghoza	*Pinus geradiana* Wall.	Fruit	UPI, Part I, Vol. VI, 46	50 g
16.	Maghz-e-Narjeel	*Cocos nuciferra* L.	Endosperm	API, Part I, Vol. III, p. 198	50 g
17.	Arq-e-Babuna	*Matricaria chamomilla* L.	Flower	UPI, Part I, Vol. II, p. 39	50 g
18.	Mawaeez Munaqqa	*Vitis vinifera* L.	Fruit	UPI, Part I, Vol. IV, 96	150 g
19.	Asal	Honey	As such	UPI, Part II, Vol. I, p.82	2.5 Kg

Drug preparation :

The formulated drug was prepared in there different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM, Part-IV, Ist Edition (Anonymous, 2006) and NFUM. Part-IV, IInd Edition (Anonymous, 2022). The required quantities of all the ingredients were taken of pharmacopoeial quality. All the ingredient were cleaned and dried, except no.8, 14 & 19 under shade to remove the moisture if any, Powdered the ingredients no.1-7 and 9-13; sieve them through mesh no. 80. Powdered the ingredient no.8 and soaked it in Arq-e-Gulab; grind it using mortar &pestle until it becomes fine paste. Powdered

coarsely ingredient no.15 using mortar and pestle and keep it separately, Powdered coarsely the ingredient no.16; sieve it through mesh no.80, Grinded the ingredient no.18 by adding honey to make a paste. Dissolved ingredient no.14 in ingredient no.17 by slow heating. At the boiling stage, add 0.1% *Citric Acid*; mix well and prepared the qiwam of 75% (Brix) consistency. Add ingredient no.19 and mix thoroughly. Removed the vessel from fired; immediately add the ingredient no. 15, 16 &18; mixed thoroughly. Finally add ingredient no.1-13, mix again thoroughly to prepared the homogenous product. Allowed the contented to cool to room temperature and store the prepared drug in a tightly closed food grade glass / plastic container free from moisture.

Pharmacopoeial standard parameters:

Pharmacopoeial research studies such as organoleptic characters, microscopical, macroscopical and physicochemical, TLC/HPLC., quality control and quality assurance parameters were carried out

1. **Organoleptic Evaluation:** Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc., using the sensory organs of our body. The organoleptic characters of the drugs samples were carried out based on the method described by Siddique *et al*. (1995).

2. **Powder Microscopy :**Since the drug contains more than 10 ingredients (total 19 ingredients), the powder microscopic study is not required. (Wallis, 1987; Johansen, 1940).

3. **Physico-chemical analysis:** If the water content is high the drug can easily be deteriorated due to fungus, The ash content indicates the total amount of inorganic material after complete incineration and the acid insoluble ash is an indicative of silicate impurities might be due to improper washing of the drug. The alcohol and water soluble extractive matter indicates the amount of bioactive chemical constituents in a given amount of particular drug when extracted with respective solvent. Some of the useful tools in standardization of ASU herbal products such as moisture content of the powdered sample at 105ºC, ash values, acid insoluble ash, solubility in water and alcohol, pH values and bulk density and estimation of sugar etc., are useful tools were studies as per standard methods (Anonymous,1987;1998).

4. **TLC/HPTLC finger printing analysis:** The drug samples (2gm) were soaked in chloroform and alcohol separately for 18 hours and refluxed for ten minutes on water bath and filtered through What man N0.1 filter paper. The filtrates were concentrated and made up to 10 ml in volumetric flask with respective solvents (Saxena and Yadav,1983). TLC/HPTLC finger print studies of chloroform and alcohol extracts of the drug were carried out using aluminium plate precoated with silica gel 60 F254 (E. Merck) with CAMAG Linomat IV sample applicator. The chromatograms of both the extracts were taken using the solvent systems

toluene: ethyl acetate (8 : 2 or 9 : 1) and toluene: ethyl acetate (8 : 2 or 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 and 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. (Khan *et al*.,2022 ; Sagar *et al*.,2020 and Wagner and Blad, 1996; Sethi, 1996).

5. Quality assurance and quality control parameters:

Estimation of microbial load: The microbial load viz. total bacterial count (TBC), total fungal count (TFC), Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aurous* 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 as per Guidelines of WHO. Heavy metals were analyzed by Atomic Absorption Spectroscopy (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 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: Aflatoxins B1, B2, G1 and G2 were analyzed as per Official Analytical Methods of the American Spice Trade Association (ASTA), 1997. Aflatoxins were estimated by Kobra cell techniques using Agilent HPLC and CAMAG or Anchrom HPTLC instruments as per the method ASTA (Anonymous, 1997; Sagar *et al*.,2020).

Details of instrument and operating parameters High Performance Liquid Chromatography (Thermo Fisher) and CAMAG or Anchrom HPTLC were 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 run: 20 µl (Aflatoxins B1, B2, G1 and G2 mixture and test samples).

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- Thermo Scientific, Model -TSQ9000 or Agilent), detector-mass selective detector or Triple Quadrupole mass analyzer detector, column specification-DB-5MS or TG-5MS, carrier gas - helium, flow rate - 1ml/min, column length - 30 m, internal diameter - 0.25 mm, column thickness - 0.25 ìm).

The usage of ASU. herbal products along with higher safety margins, WHO has taken necessary steps to ensure quality assurance and quality control parameters with the modern techniques and application of suitable standards, (Anonymous,1998;Sagar *et al*., 2020; Meena et al., 2016).

III. RESULTS AND DISCUSSION

Organoleptic character of the formulated drug Majoon-e-Maddat-ul-Hayat Jadwari indicates that the drug is dark brown in colour, having aromatic odour and pungent taste. The physico-chemical analysis such as LOD/Moisture contain obtained in the drug was ,w/w- (6.42%,6.69%,6.56%) shows the amount of moisture content present in the drug. Total ash, w/w- (1.66%,1.78%,1.66%) and Acid in-soluble ash, w/w- (0.266%,0.323%,0.318%) ,Traces amount, indicate the presence of inorganic and metals form of substances, pH(1% solution) (4.58,4.55,4.51), and pH(10% solution) (5.45,5.57,5.74) indicate the presence nature of drug Acidic or Alkaline in nature. Water soluble extractive matter, (WSEM). w/v- (73.48%, 74.01%,74.41%), Alcohol soluble extractive, (ASEM). w/v- (53.66%,54.66%,55.04%) and Alcohol soluble successive extractive value (ASSEV). w/v- 54.92%,54.96%,54.90% , Chloroform soluble successive extractive value (CSSEV). w/v- 16.85%,16.72%,16.82% the run samples obtained as yields. Water soluble extractive values (WSEM), has been indicated the presence of bioactive chemical constituents of inorganic and more polar organic bio-active phytochemical constituents content and the alcohol and chloroform soluble extractive values (ASEM) and (ASSEV), (CSSEV) have been indicated by the extraction of polar bio-active phytochemical constitutes. Bulk density gm/ml-(1.525,1.515,1.518) indicated density of the drugs, Reducing Sugar (54.31, 55.40, 54.80) and Non-Reducing Sugar (23.43,23.52, 23.50) indicated the potency of invert or non-invert Sugar (Sucrose or Fructose) concentrations in the drug samples. Semi solid formulated compound drugs analysed parameters were revealed after validation of Pharmacopeial standard parameters of Majoon-MEMUHJ a semi solid form drug shown in (Table-1) respectively.

HPTLC / Thin Layer Chromatography:

In TLC/ HPTLC finger printing profiling, After leaching out the sugar, reflux 2g of drug with 40ml of Chloroform and Ethanol separately for 30 minutes and filter. Concentrate the filtrate up to 10 ml (approx.) on water bath and applied the Chloroform extract on precoated aluminium TLC plate of silica gel 60 F_{254}using HPTLC automatic sample applicator. Develop the plate in Toluene - Ethyl acetate (8: 2)solvent system. Allow the plate to dry in air and examine under UV (366nm).Observe 09 major fluorescent spots at R_f 0.12(blue), 0.17(olive green), 0.33, 0.37(red), 0.41(blue), 0.43(light blue), 0.46, 0.50(red) & 0.61(blue). Under UV (254nm), observe 06 major spots at R_f 0.15, 0.17, 0.25, 0.31, 0.61 & 0.63(green).Dip the plate in 1% Vanillin – Sulphuric acid reagent followed by heating at 105^0C for 5 minutes and examine under visible light. Observe 08 major spots at R_f 0.13(yellow), 0.15, 0.20(bluish grey), 0.32(purple), 0.41, 0.53, 0.59 & 0.61(bluish grey). shown in (Fig.-1 and Table-2) respectively .

After applied of above said extraction processed, cconcentrated the filtrate up to 10 ml (approx.) on water bath and applied the Ethanol extract on precoated aluminium TLC plate of silica gel 60 F_{254}using HPTLC automatic sample applicator. Develop the plate in Toluene - Ethyl acetate (8: 2) solvent system. Allow the plate to dry in air and examine under UV (366nm).Observe 09 major fluorescent spots at R_f 0.18(blue), 0.28(light blue), 0.36(brown), 0.61(purple), 0.63(red), 0.66 (turquoise blue), 0.73(red), 0.76(blue) & 0.79(red). Under UV (254nm), observe 04 major spots at R_f 0.16, 0.28, 0.42, 0.47(green).Dip the plate in 1% Vanillin - Sulphuric acid reagent followed by heating at 105^0C for 5 minutes and examine under visible light. Observe 06 major spots at R_f 0.28(green), 0.37(bluish grey), 0.50(purple), 0.53, 0.62 & 0.84(bluish grey). shown in (Fig.-2 and Table-3) respectively.

Table-1: Physico-chemical parameters

Parameters Analyzed	Batch Numbers (Average Redding)		
	I	II	III
Extractives, w/v			
Water soluble matter	73.48%	74.01%	74.41%
Alcohol soluble matter	53.66%	54.22%	55.04%
Ash values, w/w			
Total ash	1.66%	1.78%	1.66%
Acid insoluble ash	0.266%	0.323%	0.343%
pH values			
1% Aqueous solution	4.58	4.55	4.51
10% Aqueous solution	5.45	5.57	5.75
LOD./ Moisture content,	4.33%	4.42%	4.32%

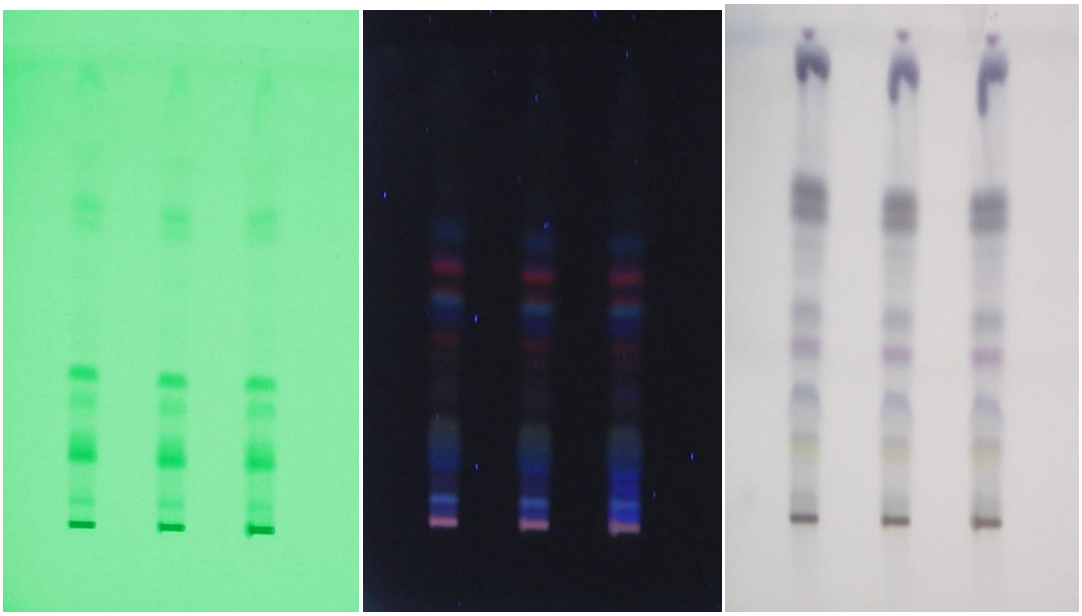
w/w			
Alcohol soluble successive extractive values (Soxhlet-Hot extraction),w/v	54.92%	54.96%	54.90%
Chloroform soluble successive extractive values (Soxhlet-Hot extraction),w/v	16.85%	16.72%	16.82%
Bulk density, gm/ml	1.525	1.515	1.518
Reducing Sugar	54.31	55.40	54.80
Non-Reducing Sugar	22.43	23.52	22.65

Table-2: R_f values of chloroform extract

Solvent system	R_f Values		
	254nm	366nm	VS reagent
Toluene : Ethyl acetate : (8 : 2)	0.15(Green)	0.12(Blue)	0.13(Yellow)
	0.17(Green)	0.17(Olive green)	0.15(Bluish grey)
	0.25(Green)	0.33(Red)	0.20(Bluish grey)
	0.31(Green)	0.37(Red)	0.32(Purple)
	0.61(Green)	0.41(Blue)	0.41(Bluish grey)
	0.63(Green)	0.43(Light blue)	0.53(Bluish grey)
		0.46(Red)	0.59(Bluish grey)
		0.50(Red)	0.61(Bluish grey)
		0.61(Blue)	

Table-3: R_f values of alcohol extract

Solvent system	R_fValues		
	254nm	366nm	VS reagent
Toluene : Ethyl acetate (8 : 2)	0.16 (Green)	0.18(Blue)	0.28(Green)
	0.28 (Green)	0.28(Light blue)	0.37(Bluish grey)
	0.42(Green)	0.36(Brown)	0.50(Purple)
	0.47(Green)	0.61(Purple)	0.53(Bluish grey)
		0.63(Red)	0.62(Bluish grey)
		0.66(Turquoise blue)	0.84(Bluish grey)
		0.73(Red)	
		0.76(Blue)	
		0.79(Red)	



B-I B-II B-III **B-I B-II B-III** **B-I B-II B-III**

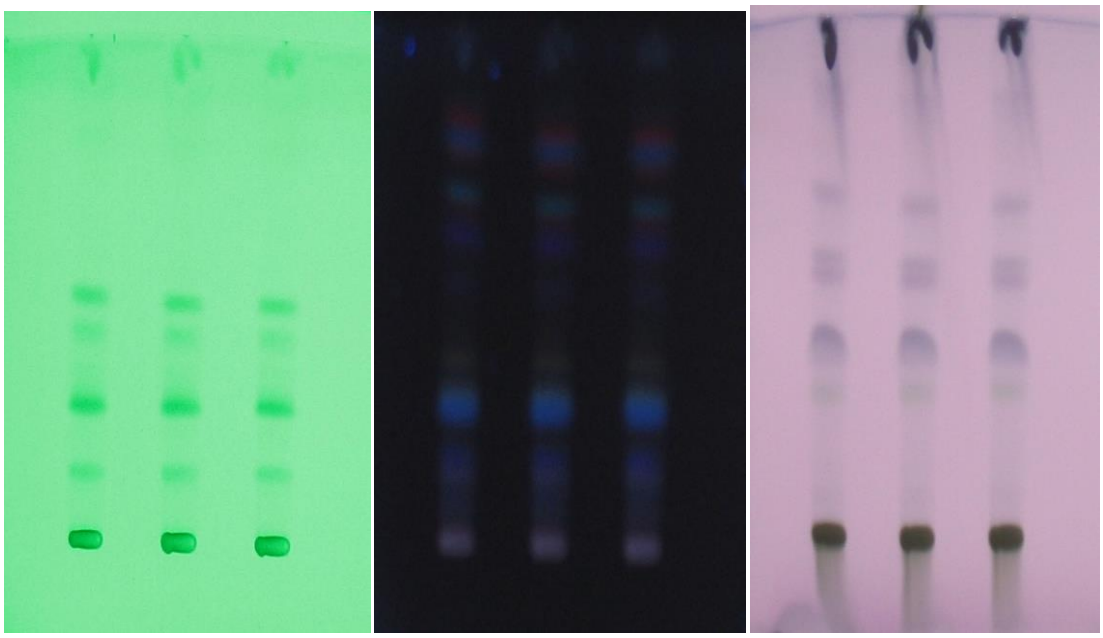
UV 254 nm UV366 nm Visible Light

(After derivatization)

Solvent System: Toluene : Ethyl acetate (8 : 2)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III

Figure 1: TLC/HPTLC Photo of Chloroform Extract



B-I B-II B-III **B-I B-II B-IIIB-I B-II B-III**

UV 254 nm UV366 nm Visible Light

(After derivatization)

Solvent System: Toluene : Ethyl acetate (8 : 2)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III

Figure 2: TLC/HPTLC Photo of Alcohol Extract

Table-4: Analysis of Microbial load

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Total Bacterial Count	700 cfu/gm	10^5cfu/gm
2	Total Fungal Count	300 cfu/gm	10^3cfu/gm
3	*Escherichia coli*	Absent	Absent
4	*Salmonella typhai Spp.*	Absent	Absent
5	*Staphylococcus aurous*	Absent	Absent

Table-5: Estimation of Heavy Metals

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Lead	3.72ppm	10ppm
2	Cadmium	0.04ppb	0.3ppm
3	Mercury	Not detected	1.0ppm
4	Arsenic	0.09 ppm	3.0ppm

Table-6: Estimation of Aflatoxins

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Aflatoxins, B1	Not detected	0.5ppm
2	Aflatoxins, B2	Not detected	0.1ppm
3	Aflatoxine, G1	Not detected	0.5ppm
4	Aflatoxine, G2	Not detected	0.1ppm

Table-7: Estimation of Pesticide Residues

S.N0.	Parameter Analyzed	Results	WHO Limit (mg/kg)
1	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	Not detected	1.0
2	HCH (sum of all isomers)	Not detected	0.3
3	Endosulphan (all isomers)	Not detected	3.0

4	Azinphos-methyl	Not detected	1.0
5	Alachlor	Not detected	0.02
6	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	Not detected	0.05
7	Chlordane (cis & tans)	Not detected	0.05
8	Chlorfenvinphos	Not detected	0.5
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Not detected	0.05
10	Endrin	Not detected	0.05
11	Ethion	Not detected	2.0
12	Chlorpyrifos	Not detected	0.2
13	Chlorpyrifos-methyl	Not detected	0.1
14	Parathion methyl	Not detected	0.2
15	Malathion	Not detected	1.0
16	Parathion	Not detected	0.5
17	Diazinon	Not detected	0.5
18	Dichlorvos	Not detected	1.0
19	Methidathion	Not detected	0.2
20	Phosalone	Not detected	0.1
21	Fenvalerate	Not detected	1.5
22	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	Not detected	1.0
23	Fenitrothion	Not detected	0.5
24	Deltamethrin	Not detected	0.5
25	Permethrin (sum of isomers)	Not detected	1.0
26	Pirimiphos methyl	Not detected	4,0

IV.CONCLUSION

Standardization is an essential part for the evaluation and validation of scientific standards to justify the quality of poly herbal formulation Majoon-e-Maddat-ul-Hayat Jadwari. To maintain the batch-to-batch uniformity, consistency and quality of the drug, each plant material used in preparation of 'Majoon-e-Maddat-ul-Hayat Jadwari' was identified and evaluated for their pharmacopoeial standards. The water soluble extractive values yields (WSEM), has been indicated and provided the presence of bioactive chemical constituents of inorganic and more polar organic bio-active phytochemical constituents content and the alcohol and chloroform soluble extractive values (ASEM) and (ASSEV), (CSSEV) have been indicated and provided by the extraction of polar bio-active phytochemical constituents present in the analyzed 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. In the present investigated research studies of various analyzed data, quality standard parameters such as heavy metals, aflatoxins, pesticide residues and microbial load were found within permissible limit of WHO guidelines. Physico-chemical, TLC/HPTLC finger printing, WHO parameters were revealed and carried out which can be laid down as reference standards of the drug MEMUHJ. From the present studies it can be concluded that the formulated MEMUHJ drug is safe and free from any toxic, hazardous substance, It is an economic drug and the efficacy of the drug can be used as a traditional alternative medicine as a Nervine Tonic, referential information evaluated by conducting the clinical studies on patient suffering Amnesia, Urinary Incontinence, Renal pain /Nephralgia and Rheumatoid / Poly Arthritis disease as mentioned in the classical Unani, authenticated and NFUM Vol-IVth. Edition Ist and IInd Classical Pharmacopeial literature or text basis. The formulation MEMUHJ was standardized for the first time gives rise to various validated standard parameters. The results obtained may provide as a reference standard and could be beneficial for consideration of efficacious polyherbal Unani formulation for future research endeavours as well as can be incorporated of pharmacopoeial standard monograph.

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VI. REFERENCES

[1]. Asma Sattar Khan, Shoeb Ahmed Ansari, Sonali Sajwan, Pawan Kumar Sagar, Reesha Ahmed and Syed Arshad Ali Hashmi. 2022.Pharmacognostical and HPTLC Evaluation of Jawarish-e-Ood Tursh: An Anti-emetic Unani Formulation. European Journal of Biomedical and Pharmaceutical Sciences ; 7(12): 210-218. (ISSN: 2349-8870); DOI: https://www.ejbps.com/ejbps/abstract_id/8544

[2]. Anonymous.2022. National Formulary of Unani Medicine Part IV, Edition -IInd, published by PCIM&H, Ghaziabad UP, Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Government of India, New Delhi.:60; DOI: https://pcimh.gov.in/WriteReadData/RTF1984/1536753955.pdf

[3]. Naaz A.,Viquar U., Naikodi M.A.R., Siddiqui J.I., Zakir M., Kazmi M.H., Minhajuddin A.2021.Development of Standard Operating Procedures (SOPs), Standardization, TLC and HPTLC Fingerprinting of a Polyherbal Unani Formulation. Journal of Cell Med; 11(4);e21:1-9. DOI: 10.5667/CellMed.2021.0021

[4]. Pawan Kumar Sagar, R Murugeswaran, R.P. Meena, M.W. Ahmed, S.A. Ansari, S. Khair and S A A Hashmi.2020.Standardization and HPTLC Finger Printing Studies of Poly Herbal Formulation - Itrifal Haamaan. International Journal of Ayurveda and Pharmaceutical Chemistry;12(3):220-232.(e-ISSN:2350-0204); DOI: https://oaji.net/articles/2021/1791-1632827247.pdf

[5]. Pawan Kumar Sagar, R Murugeswaran, R.P. Meena, A.S. Khan, S. Mageswari, P.M.D. Sri, S. Sajwan, S.G. Ahmad.2020.Standardization and Monographs development, HPTLC .Finger printing research studies of Poly Herbal Formulation-Habb-e-Nishat Jadeed. European Journal of Biomedical and Pharmaceutical Sciences ; 7(12): 210-218. (ISSN: 2349-8870);DOI: https://storage.googleapis.com/journal-uploads/ejbps/article_issue/volume_7_december_issue_12/1606466607.pdf

[6]. Sagar, P.K., Murugeswaran, R., Meena, R., Mageswari, S., and Sri, P.Meera Devi, Khair, S.2020. Standardization and HPTLC. Fingerprinting study of Poly Herbal Unani Formulation-Habb-e-Sara Khas. International Journal of Traditional and Complementary Medicine;5(21),1-13.;DOI: https://escipub.com/ijtcm-2020-01-2805/

[7]. Meena, R.P., Verma, S.C., Mageswari, S., Sri Devi, P.M., Khan, A.S., Ansari, S.A., Arfin ,S. ,Aminuddin.2016. Scientific validation of Unani Formulation : Majoon-e-Hamal Ambari Alwi Khani. Hippocratic Journal of Unani Medicine;11(1):47-60. DOI: https://www.ccrum.res.in/writereaddata/UploadFile/Hippocratic13_1792.pdf

[8]. Bahuguna Y, Zaidi S, Neeraj Kumar and Kailash Rawat.,2014. Standardization of Polyherbal Marketed Formulation Triphala Churna, Research and Review. Journal of Pharmacognosy and Phytochemistry; 2 (3):28-35.

[9]. Kunle, O.F., Egharevba, H.O., Ahmadu, P.O. 2012. Standardization of Herbal Medicines :The Review . Int. J Biodivers. Conserve ;4:101-112.

[10]. Yadav P, Mahour Y and Ashok Kumar.2011. Standardization and Evaluation of Herbal Formulations. Journal of Advanced Laboratory Research in Biology;2(4):161-166.

[11]. Anonymous. 2011. National Formulary of Unani Medicine, Part-VI, Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi,(2.19):75.

[12]. Anonymous. 2009. The Unani Pharmacopeia of India, Part-I, Vol-VI, published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi:31-32 ,40-41,68-69 ,72-73 and 88-89.

[13]. Ramasamy D., Meena R.P., Khan S.A., Arfin S., Magaswari S., Sultana G. 2009. Chemical Examination of Majoon-e-Rewand – A Unani formulation. Hippocratic Journal of Unani Medicine;4(1):59-67.

[14]. Anonymous. 2009. The Unani Pharmacopeia of India, Part-I, Vol-VIth, published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi: 31;46.

[15]. Anonymous. 2009. The Unani Pharmacopeia of India, Part-I, Vol-Vth, published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi: 46; 80-81,105;109.

[16]. Anonymous. 2007. The Ayurvedic Pharmacopeia of India, Part-I, Vol-IVth,

published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi :105.

[17]. Anonymous.2007. The Unani Pharmacopoeia of India, Part-I, Vol.-I, Govt. of India, Min. of Health & Family Welfare, New Delhi: 5-6,17; 26,78 ;80 and 88.

[18]. Anonymous. 2007.The Unani Pharmacopoeia of India, Part-I, Vol.-II, Govt. of India, Min. of Health & Family Welfare, New Delhi:29;39,45-46, 51-52,81-82, 89-90 and 91-92.

[19]. Anonymous. 2007.The Unani Pharmacopoeia of India, Part-I, Vol.-III, Govt. of India, Min. of Health & Family Welfare, New Delhi:23;74 and 88.

[20]. Anonymous. 2007. The Unani Pharmacopeia of India, Part-I, Vol.-IV, Govt. of India, Min. of Health & Family Welfare, New Delhi: 38,49-50.

[21]. Anonymous. 2006, Official Methods of Analysis(AOAC), International, Horwitz W, Latimer G W,18thEdn. AOAC International; chapter 26,:17.

[22]. Patel, P.M., Patel, N.M., Goyal, R.K.,2006. Quality control of herbal products, The Indian Phamacist,5(45):26-30.

[23]. Hkm. Mohammad Hadi Hussain Khan Muradabadi.2005.Ilaj-al-Amraz (Urdu)., CCRUM, Ministry of AYUSH, Govt. of India: 714.

[24]. Anonymous. 2005. Global Atlas of Traditional, Complementary and Alternative Medicine, Vol.1 and Vol. 2. WHO, Geneva. EMEA, 2005. Guidelines on Quality of Herbal Medicinal Products/Traditional Medicinal Products, EMEA/CVMP/814OO Review. London: European Agency for the Evaluation of Medicinal Products (EMEA).

[25]. Anonymous. 2005. Official Methods of Analysis of AOAC International, Horwitz W, Latimer GW(edn). 18th Edn. AOAC International: Maryland, chapter 3, p. 10-11, chapter 10 p.18-23 and chapter-26,:17.

[26]. Anonymous. 2001. The Ayurvedic Pharmacopeia of India, Part-I, Vol-III[rd], published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi :198.

[27]. Anonymous. 2001. National Formulary of Unani Medicine, Part-IV;(Ist Edition), Ministry of Health & Family Welfare Government of India, Department of Indian Systems of Medicine and Homoeopathy:52.

[28]. Anonymous. 2002. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, WHO, Geneva.

[29]. Anonymous. 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, WHO, Geneva.

[30]. Anonymous.1998. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva: 25-28.

[31]. Anonymous. 1997. Official Analytical Methods of the American Spice Trade Association (ASTA), Inc. 4thedn. New Jersey: 149-152.

[32]. Sethi, P.D., 1996. High Performance Thin Layer Chromatography, 1st Edn., Vol. X. CBS Publishers and Distributers, New Delhi:1-56.

[33]. Wagner, H and Biadi, S.,1996. Plant Drug Analysis - A Thin Layer Chromatography Atlas, Springer-Verlag, 2ndEdn., Germany.

[34]. Siddqui., Hakim M.A.1995. Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, 24-25th January (appendix), Central Council for Research in Unnai Medicine, New Delhi.

[35]. Anonymous.1991. Physico-chemical standards of Unani Formulations, Part-II, CCRUM, Min. of Health and Family Welfare, New Delhi: 15-57

[36]. Anonymous. 1990. The Ayurvedic Pharmacopoeia of India, Part –I,Vol.-1,Govt. of India, Min. of Health & Family Welfare, New Delhi: 120-121 and 142-143.
[37]. Anonymous. 1987. Physico-chemical standards of Unani Formulations, Part - II.CCRUM , Ministry of Health & Family Welfare, New Delhi: 9-54; 300-317.
[38]. Anonymous.1987. Physico-chemical standards of Unani Formulations, Part-II, CCRUM, Min. of Health and Family Welfare, New Delhi: 9-54.
[39]. Wallis, R.E., 1987. Text Book of Pharmacognosy, 5th Edition. CBS Publisher & Distributors , Delhi: 494-496.
[40]. Anonymous.1986. Physico-chemical standards of Unani Formulations, Part-II, CCRUM, Min. of Health and Family Welfare, New Delhi: 27-42
[41]. Wagner, H. and Bladt, S. 1984. Plant Drug Analysis - A Thin Layer Chromatography Atlas. Springer-Verlag, 2nd Edn., Germany.
[42]. Saxena, S.C. and Yadav, R.S.1983. A new plant extract to suppress the population of yellow fever and dengue vector Aedesaegyptii L. Current Science; 52: 713-715.
[43]. Anonymous. 1981. National Formulary of Unani Medicine, Part-I. Central Council for Research in Unani Medicine, Ministry of Health and Family Welfare, Govt. of India, p. 134.
[44]. Johansen, D.A. 1940. Plant Microtechnique. Mc. Graw Hill Book Company Inc., New York and London, pp. 181-186.
[45]. Hkm. Mohammad Sharif Khan.1921(A,D,).Ilaj-al-Amraz (Farsi):, Barqi Press, Delhi:288.

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