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Scientific Standard Validation and HPTLC. Finger printing studies of Polyherbal Unani Formulation Jawarish-e-Usquf

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ABSTRACT

Standardization and product validation is used to describe all measures under taken during the manufacturing process and quality control and quality assurance of drug leading to its reproducible quality. Therefore we need to develop standard validation techniques to standardize and validate of the herbal formulations. The drug Jawarish-e-Usquf is therapeutically useful in the treatment of Laqwa (Facial / Bell's palsy), Qulanj (intestinal pain, coli), Nafkh-i-Shikam (flatulence), Bawasir (piles / haemorrhoids), Waja' al-Khasira (low backache) Waja' al-Zahr (bachache), Qay(vomiting) Action wise Munaqqi-e-Asab, (Nervine Cleansers). The drug JEU was prepared in three different batches as per the guidelines of National Formulary of Unani Medicine(Part-IV[th]) and UPI.(Part-II[nd], Edition-II[nd]). Present research study is aimed to evaluate the product quality validation using physico-chemical parameters; HPTLC fingerprinting as per WHO guidelines of analyzed parameters. The physico-chemical average reading data's of every III Batches of test samples showed that the drug contain LOD/ Moisture ,w/w- (15.14%,14.44%, 14.63%), Total ash, w/w- (1.34%,1.37%,1.42%), Acid in-soluble ash, w/w-(0.015%,0.014%,0.016%), Alcohol and water soluble extractive matter,(ASEM and WSEM) w/v- (60.21%, 60.54%,61.29%) & (70.78%,71.24%, 70.58%),and (ASSEV.), (CSSEV.) extractive values w/v.- (16.81%,16.80,15.80), (7.85%, 7.87%,7.86%), pH(1% solution) (5.77,5.83,5.69), pH(10% solution) (4.66,4.81, 4.74), Bulk density ,gm/ml - (1.615,1.606,1.603),Reducing Sugar (53.78, 53.36, 52.97) and Non-Reducing Sugar (6.32,6.79,6.27) the HPTLC finger prints showed various spots at 254nm, 366nm and visible light (V-S reagent).The validation of quality control studies revealed the absence of Microbial load, Aflatoxins, Heavy metal and Pesticide residues, The evaluated validated quality standards will be very useful for referential support, validation of the standards of JEU formulated drug, pharmaco-vigilance and providing the quality medicine to needful human being.

Keywords: Jawarish-Usquf, Physico-chemical quality, Quality Control and Assurance parameters, TLC/ HPTLC fingerprinting and Unani Compound drug.

I. INTRODUCTION

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).

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. All medicines, either synthetic or plant origin, have to fulfil 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)

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)

The drug Jawarish-e-Usquf, is one the classical Unani poly-herbal compound formulation. It is therapeutically useful in the ailment like Laqwa (Facial / Bell's palsy), Qulanj (intestinal pain, coli) and Bawasir (piles / haemorrhoids). The Unani drug standardization research studies of the drug JEU has been 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 as Munaqqi-e-Asab, (Nervine Cleansers) and Laqwa (Facial / Bell's palsy), Qulanj (intestinal pain, coli), Nafkh-i-Shikam (flatulence), Bawasir (piles / haemorrhoids), Waja' al-Khasira (low backache) Waja' al-Zahr (bachache), Qay(vomiting). Jawarish-Usquf is a dark brown semi-solid preparation with characteristic of its own smell form preparation with agreeable, aromatic odour and sweetish-bitter taste. JEU was reported bioactive to contain active phytochemical constituents such as Alkaloids, Glycosides, Tannins, Crude fibres etc. The preparation of the drug in different batches is based on traditional methods in accordance with the procedure given in NFUM, Part-IVth, Ist Edition (Anonymous, 2006) and NFUM. Part-IVth, IInd Edition (Anonymous, 2022).

II. METHODS AND MATERIAL

Ingredients used for preparation:

The raw drug formulation is composed of the following mention ingredients:

Sr. No.	Unani Name	Botanical/ English Name	Part Used	Reference	Qty.
1.	Zanjabeel Khushk,	*Zingiber officinale* Rosc.	Root	UPI, Part I, Vol. I, p. 88,	10g
2.	Dalchini,	*Cinnamomum zeylanicum*. Blume	Stem Bark	UPI, Part I, Vol. I, p. 26,	10g
3.	Aamla Munaqqa,	*Emblica officinalis* Gaertn.	Fruit	UPI, Part I, Vol. I, p.5-6,	10g
4.	Qaranful,	*Syzygium aromaticum*, Merr & L.M. Perry	Flower bud	UPI, Part I, Vol. I, p.70,	10g
5.	Bisfayej,	*Polypodium vulgare* L.	Rhizomes	UPI, Part I, Vol. II, p.29,	10g
6.	Jauzbuwa,	*Myristica fragrans* Houtt.	Seeds	UPI, Part I, Vol. I, p. 38,	10g
7.	Filfil Siyah,	*Piper nigrum* L.	Fruit	UPI, Part I, Vol.IV, P. 38,	15g
8.	Heel Kalan	*Amomum subulatum* Roxb.	Fruit	UPI, Part I, Vol. IV, p. 49,	15g
9.	Saqmonia,	*Convolvulus scammonia* L.	Resin	---- ,	15g
10.	Turbud,	*Operculina turpethum* (L) Silva Manso	Root	UPI, Part I, Vol. V, p. 105,	15g
11.	Qand Safaid,	Cane sugar	Crystal	---- ,	15g

Drug preparation :

The formulated drug was prepared in there different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM, Part-IVth, Ist Edition (Anonymous, 2006) and NFUM. Part-IVth, IInd Edition (Anonymous, 2022). The required quantities of all the ingredients were taken of pharmacopoeial quality. All the ingredient were cleaned and dried, ingredient no.1-10 under shade to remove the moisture if any powdered the ingredient number 1 &2 and 4-10 separately, sieved through 100 mesh and kept separately and mixed all the powders thoroughly. Grind the ingredient number 3 to make the paste and keep separately, Dissolved ingredient no.11 in 375 ml of water by slow heating. At the boiling stage, add 0.1% *Citric Acid*; mixed well and prepared the qiwam of 75% (Brix) consistency. Added 0.1% Sodium Benzoate and the paste of ingredient no. 3; mixed well and re-corrected the qiwam up to 77% (Brix) consistency, Remove the vessel from the fire; immediately add the mixed powder of ingredients no. 1 & 2 and 4-10; mixed thoroughly to prepare the homogenous product, Allowed the content to cool to room temperature and stored 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 :** Take 3-5g powder drug sample was weighed, mixed with 50ml of distil water in a beaker and warmed gently in order to make complete dispersion in water. Then mixture was centrifuged and decanted supernatant. The sediment were washed several times with distilled water, centrifuged again and decanted the supernatant. Small quantity of the sediment was taken and mounted in glycerine, 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 glycerine to locate lignified cells. The following characters in different mounts were observed (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 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 105^{o}C, 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 indicates that the drug is dark brown colour, aromatic odour and pungent taste. The physico-chemical analysis such as LOD/Moisture contain obtained in the drug was ,w/w- (15.14%,14.44%, 14.63%) shows the amount of moisture content present in the drug. Total ash, w/w- (1.34%,1.37%,1.42%) and Acid in-soluble ash, w/w-(0.015%,0.014%, 0.016%) ,Traces amount, indicate the presence of inorganic and metals form of substances, pH(1% solution) (5.77,5.83,5.69), and pH(10% solution) (4.66,4.81,4.74) indicate the presence nature of drug Acidic or Alkaline in nature. Water soluble extractive matter, WSEM., w/v.- (70.78%,71.24%,70.58%) and Alcohol soluble extractive matter, ASEM., w/v.- (60.21%, 60.54%, 61.29%), and (ASSEV.), (CSSEV.) extractive values w/v.- (16.81%,16.80,15.80), (7.85%, 7.87%,7.86%) the run samples obtained as Water soluble extractive matter (WSEM), w/v.- yields indicated the presence of inorganic and more polar organic bio-active phytochemical constituents content and the alcohol soluble extractive matter, alcohol and chloroform soluble successive extractive values, (ASEM and (ASSEV.), (CSSEV.),w/v.- indicated in the extractions presence of polar bio-active phytochemical constitutes constituents., Bulk density gm/ml- (1.615,1.606,1.603) indicate density of the drugs, Reducing Sugar (53.78, 53.36, 52.97) and Non-Reducing Sugar (6.32, 6.79, 6.27) indicate the potency of invert or non-invert Sugar concentrations in the drug samples. Semi solid formulated compound drugs analysed parameters

were revealed of validation of Pharmacopeial standard parameters of Jawarish a semi solid form drug shown in (Table-1) respectively.

HPTLC / Thin Layer Chromatography:

TLC/ HPTLC finger printing profiling of chloroform extract of 2g of sample with 20ml of chloroform separately and reflux on water bath for 30min. Filter and Concentrate the filtrate up to 10 ml (approx.) on water bath and apply 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 (254nm), observe 09 major spots at R_f 0.11, 0.16, 0.21, 0.27, 0.32, 0.44, 0.64, 0.78 & 0.84(green).and examine under UV (366nm).Observe 11 major fluorescent spots at R_f0.12, 0.14(light blue), 0.18(blue), 0.21, 0.23(olive green), 0.33(blue), 0.35, 0.37(red), 0.50(light blue), 0.58(red) & 0.72(blue). Dip the plate in 1% *Vanillin – Sulphuric acid* reagent followed by heating at 105^{0}C for 5 minutes and examine under visible light. Observe10 major spots at R_f 0.14(bluish grey), 0.17(olive green), 0.28, 0.33(bluish grey), 0.37(purple), 0.42, 0.54, 0.64(bluish grey), 0.68(yellow) & 0.75(purple). shown in (Fig.-1 and Table-2) respectively .

Same 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 (254nm), observe 07 major spots at R_f0.14, 0.27,0.30, 0.35, 0.41, 0.47 & 0.61(green).and examine under UV (366nm).Observe 10 major fluorescent spots at R_f0.22, 0.26(blue), 0.28, 0.35(olive green), 0.47(blue), 0.55(red), 0.61(light blue), 0.68(red), 0.71(blue) & 0.75(red). Dip the plate in 1% *Vanillin – Sulphuric acid* reagent followed by heating at 105^{0}C for 5 minutes and examine under visible light. Observe 06 major spots at R_f 0.26(green), 0.33, 0.35(bluish grey), 0.47(purple), 0.60(bluish grey)& 0.78(purple). shown in (Fig.-2 and Table-3) respectively.

Table-1: Physico-chemical parameters

Parameters Analyzed	Batch Numbers		
	I	II	III
Extractives, w/v Water soluble matter, (WSEM) Alcohol soluble matter, (ASEM)	 23.09% 20.38%	 23.12% 20.41%	 23.09% 20.38%
Ash values, w/w Total ash Acid insoluble ash	 7.70% 4.32%	 7.70% 4.32%	 7.70% 4.32%
pH values 1% Aqueous solution 10% Aqueous solution	 5.58 5.66	 5.60 5.67	 5.60 5.67
LOD./ Moisture content, w/w	2.32%	2.34%	2.33%
Alcohol soluble successive extractive values,(ASSEV.), (Soxhlet-Hot extraction),w/v	16.81%	16.80%	15.80%
Chloroform soluble successive extractive values, (ASSEV.), (Soxhlet-Hot	7.85%	7.87%	7.86%

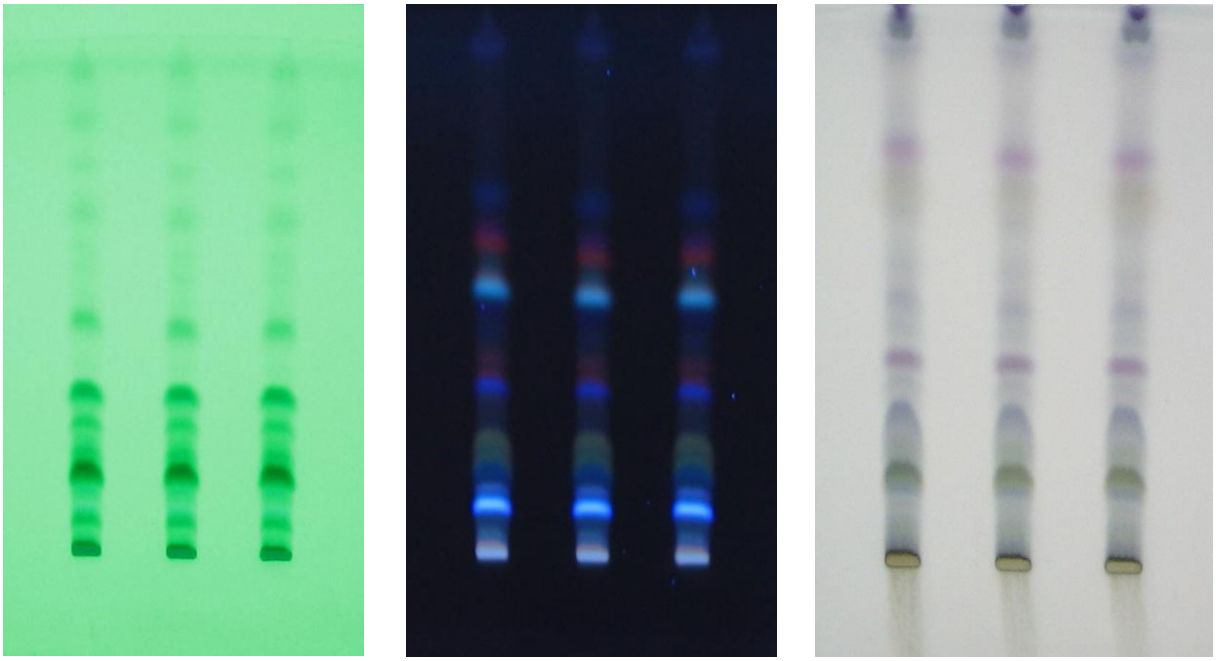
extraction),w/v			
Bulk density, gm/ml	0.5492	0.5413	0.5498
Reducing Sugar	53.78	53.36	52.97
Non-Reducing Sugar	6.32	6.79	6.27

Table-2: *Rf* values of chloroform extract

Solvent system	*Rf* Values		
	254nm	**366nm**	**VS reagent**
Toluene : Ethyl acetate : (8 : 2)	0.11(Green)	0.12(Light blue)	0.14(Bluish grey)
	0.16(Green)	0.14(Light blue)	0.17(Olive green)
	0.21(Dark green)	0.18(Blue)	0.28(Bluish grey)
	0.27(Green)	0.21(Olive green)	0.33(Bluish grey)
	0.32(Green)	0.23(Olive green)	0.37(Purple)
	0.44(Dark green)	0.33(Blue)	0.42(Bluish grey)
	0.64(Green)	0.35(Red)	0.54(Bluish grey)
	0.78(Light Green)	0.37(Red)	0.64(Bluish grey)
	0.84(Light Green)	0.50(Light blue)	0.68(Yellow)
		0.58(Red)	0.75(Purple)
		0.72(Blue)	

Table-3: *Rf* values of alcohol extract

Solvent system	*Rf* Values		
	254nm	**366nm**	**VS reagent**
Toluene : Ethyl acetate (8 : 2)	0.14 (Green)	0.22(Blue)	0.26(Green)
	0.27 (Green)	0.26(Blue)	0.33(Bluish grey)
	0.30(Green)	0.28(Olive green)	0.35(Bluish grey)
	0.35(Green)	0.35(Olive green)	0.47(Purple)
	0.41(Green)	0.47(Blue)	0.60(Bluish grey)
	0.47(Green)	0.55(Red)	0.78(Purple)
	0.61(Green)	0.61(Light blue)	
		0.68(Red)	
		0.71(Blue)	
		0.75(Red)	



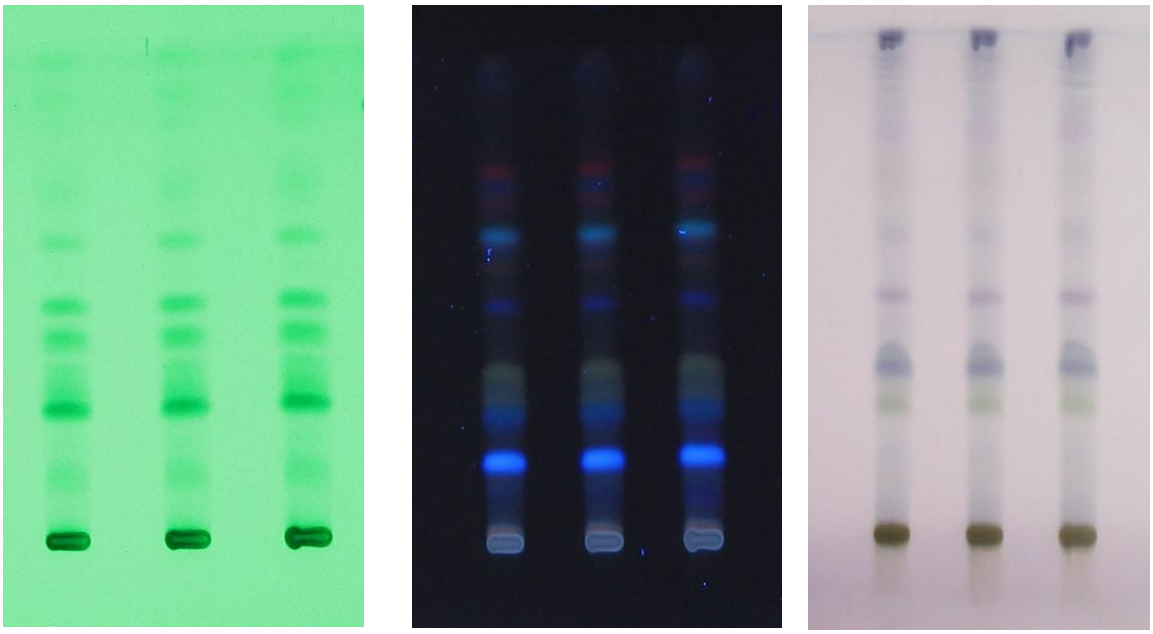
B-I B-II B-III
UV - 254nm

B-I B-II B-III
UV - 366nm

B-I B-II B-III
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
UV – 254nm

B-I B-II B-III
UV - 366nm

B-I B-II B-III
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

Quality Assurance and Quality Control Parameters:

Detection and validation of Pharmacopeial quality parameters of test samples in order to assess the quality of drug samples. The analysis of microbial load present in the drug showed that the total bacterial count (TBC) and total fungal count(TFC) was revealed 600 and 500 cfu/gm. The detection of the microbial load was under the permissible limits of WHO guideline. the estimation of microbial load viz. total bacterial count (TBC), total fungal count (TFC), *Enterobacteriaceae*, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aurous* were analyzed and found to be in permissible limit. The results are shown in (Table - 4). 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. The results are shown in (Table-5). The studies of other parameters like estimation of aflatoxins such as B1, B2, G1 and G2 The results are shown in (Table- 6) and pesticide residue such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion etc. were not detected from the drug, The results are shown in (Table -7) respectively.

Table-4: Analysis of Microbial load

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Total Bacterial Count	600 cfu/gm	10^5cfu/gm
2	Total Fungal Count	500 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	2.52ppm	10ppm
2	Cadmium	0.03ppb	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	Aflatoxine, B1	Not detected	0.5ppm
2	Aflatoxine, 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 formulations. To maintain the batch-to-batch uniformity, consistency and quality of the drug, each plant material used in preparation of 'Jawarish-Usquf' was identified and evaluated for their pharmacopoeial standards. 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 can be laid down as reference standards of the drug JEU. From the present studies it can be concluded that the formulated JEU 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 Cleansers, referential information evaluated by conducting the clinical studies on patient suffering Facial / Bell's palsy, intestinal pain, coli , piles / haemorrhoids and vomiting disease as mentioned in the classical Unani, authenticated text and NFUM Pharmacopeial literature or text basis. Can be incorporated of pharmacopoeial standard monograph.

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