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Original Article

Development of SOP's and pharmacopoeial standards for Jawarish-e-Jalinoos

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ABSTRACT

Background: Jawarish-e-Jalinoos is a Unani poly herbal compound formulations listed under the Majooniath category in National Formulary of Unani Medicine (NFUM), Part-I. It is used in various ailments such as Zof-e-Aza-Raeesa (weakness of the principle organs like brain, heart and liver), Zof-e-Meda (weakness of the stomach), Nafkh-e-Shikam (flatulence in the stomach) and Khafqan (palpitation).

Aim: Present study was undertaken to develop the Standard Operating Procedure for the preparation of drug Jawarish-e-Jalinoos and to evaluate its pharmacopoeial standards.

Methods: To prepare the drug in different batches at laboratory scale using 18 single drugs were used. The drug was prepared as per the method prescribed in NFUM. To evaluate the pharmacopoeial standards and quality control parameters pharmacognostical, physico-chemical and WHO methods were followed.

Results: Microscopical studies show the presence of various characteristic features of single drugs used in the preparation viz., vessels elements with reticulate, scalariform, pitted; perisperm cells with angular and bulbous projections; pollen grains; fibre like sclereids; septate fibres; starch grains of various shapes and sizes; stone cells with horse shoe shaped thickening; druses of calcium oxalate crystals. The physico-chemical study shows presence of moisture content 19.87%, ash content 0.8% and acid insoluble ash 0.20%. Alcohol and water soluble extractive value of the drug obtained were 40.80% and 62.78% respectively. Other parameters such as heavy metals, microbial load, aflatoxins and pesticidal residues were found within the permissible limit.

Conclusion: The evaluated data from this study will be helpful in laying down the SOP's and pharmacopoeial standards for the drug Jawarish-e-Jalinoos.

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1. Introduction

The Herbal products of traditional medicines such as Unani, Ayurveda and Siddha play a major role in health care of developing world's rural population. Standards of herbal drugs relate to the uniformity in quality, which are numerical quantities by which the quality of products may be assessed.[1] Jawarish-e-Jalinoos is one of the important herbal Unani compound formulations. The herbal formulation is being used in the ailments of weakness of the principal organs (brain, heart and liver), hepatitis, flatulence in the stomach and palpitation.[2] According to formulation composition, the Jawarish-e-Jalinoos consist of 18 ingredients. As there is no scientific procedure to prepare the drug it is planned to develop the SOP's and pharmacopoeial standards. In order to lay down the SOP's and pharmacopoeial standards, the drug was prepared in three different batches in DSRU, RRIUM, Chennai and subjected for analysis. The SOP's include procurement of ingredients, authentication, removal of adulteration if any and evaluation of their pharmacopoeial standards, powdering of raw drug to the required fineness and method of preparation. The present study was an attempt to scientifically validate the drug by applying modern parameters such as microscopical, physico-chemical, thin layer chromatography and WHO parameters such as microbial load, aflatoxin, heavy metal and pesticide residue.

2. Material and methods

The raw drugs of the formulation were procured from raw drugs dealers of Chennai. The raw drugs were identified using pharmacognostical methods[3] and evaluated their pharmacopoeial standards. The drug Jawarish-e-Jalinoos was prepared in different batches at laboratory scale as per the formulation composition.

2.1. Composition of formulation

Jawarish-e-Jalinoos is a semi-solid preparation made with the following ingredients in the composition as given in Table 1.

2.2. Method of preparation

All the ingredients were taken of pharmacopoeial quality. Clean, dried and made the powders of the ingredients number 2–16 and sieved through 80 mesh and kept separately. The ingredient number 1 was slowly grinded using mortar and pestle to make the finest form of powder. The ingredient number 17 was grinded with Arq-e-Gaozaban using mortar and pestle and kept separately. The powders of ingredient number 1–16 were mixed. The required quantity of ingredient number 18 was dissolved in 700 ml of water on slow heat and boiled the content, at the boiling stage 0.1% citric acid was added and mixed well. Then the contents were boiled to prepare the 72% consistency of Quiwam and added the ingredient number 17, mixed thoroughly and recorrected the Quiwam upto 75% consistency. Then the vessel was removed from the fire. While hot condition, the mixed powders of ingredients 1–16 were added and mixed thoroughly to prepare the homogenous product. The product was allowed to cool at room temperature and packed in tightly closed containers to protect from light and moisture.

2.3. Powder microscopy

The drug sample (5 g) 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 of the sediment was taken and mounted in glycerin. Then few mg was taken in

Table 1 – List of ingredients of the Jawarish-e-Jalinoos formulation.

S. no.	Unani name	Botanical/English name	Part used	Quantity taken for SOP
1	Mastagi	*Pistacia lentiscus* Linn.	Resin	25 g
2	Sumbul-ut-Teeb	*Nardostachys jatamansi DC*	Rhizome	10 g
3	Heel Khurd	*Eletarria cardamomum* (L) Maton.	Fruit	10 g
4	Saleekha	*Cinnamomum cassia* Blume.	Stem bark	10 g
5	Darchini	*Cinnamomum zeylanicum* Blume.	Inner stem bark	10 g
6	Khulanjan	*Alpinia galanga* (L.) SW.	Rhizome	10 g
7	Qaranful	*Syzygium aromaticum* (L.) Merr. L M Perry	Flower bud	10 g
8	Sad Kufi	*Cyperus rotundus* Linn.	Rhizome	10 g
9	Zanjabeel	*Zingiber officinale* Rosc.	Rhizome	10 g
10	Filfil Daraz	*Piper longum* Linn.	Fruit	10 g
11	Filfil Siyah	*Piper nigrum* Linn.	Fruit	10 g
12	Qust Shireen	*Saussurea lappa* C. B.	Root	10 g
13	Ood-e-Balsan	*Commiphora gileadensis* (L.) C. Chr.	Wood	10 g
14	Asaroon	*Asarum europaeum* Linn.	Rhizome	10 g
15	Habb-ul-Aas	*Myrtus communis* Linn.	Fruit	10 g
16	Chiraita Shireen	*Swertia chirata* Buch. Ham.	Whole plant	10 g
17	Zafran	*Crocus sativus* Linn.	Style & stigma	10 g
18	Qand Safaid	Sugar	–	600 g

Fig. 1 – Powder microscopy of Jawarish-e-Jalinoos.

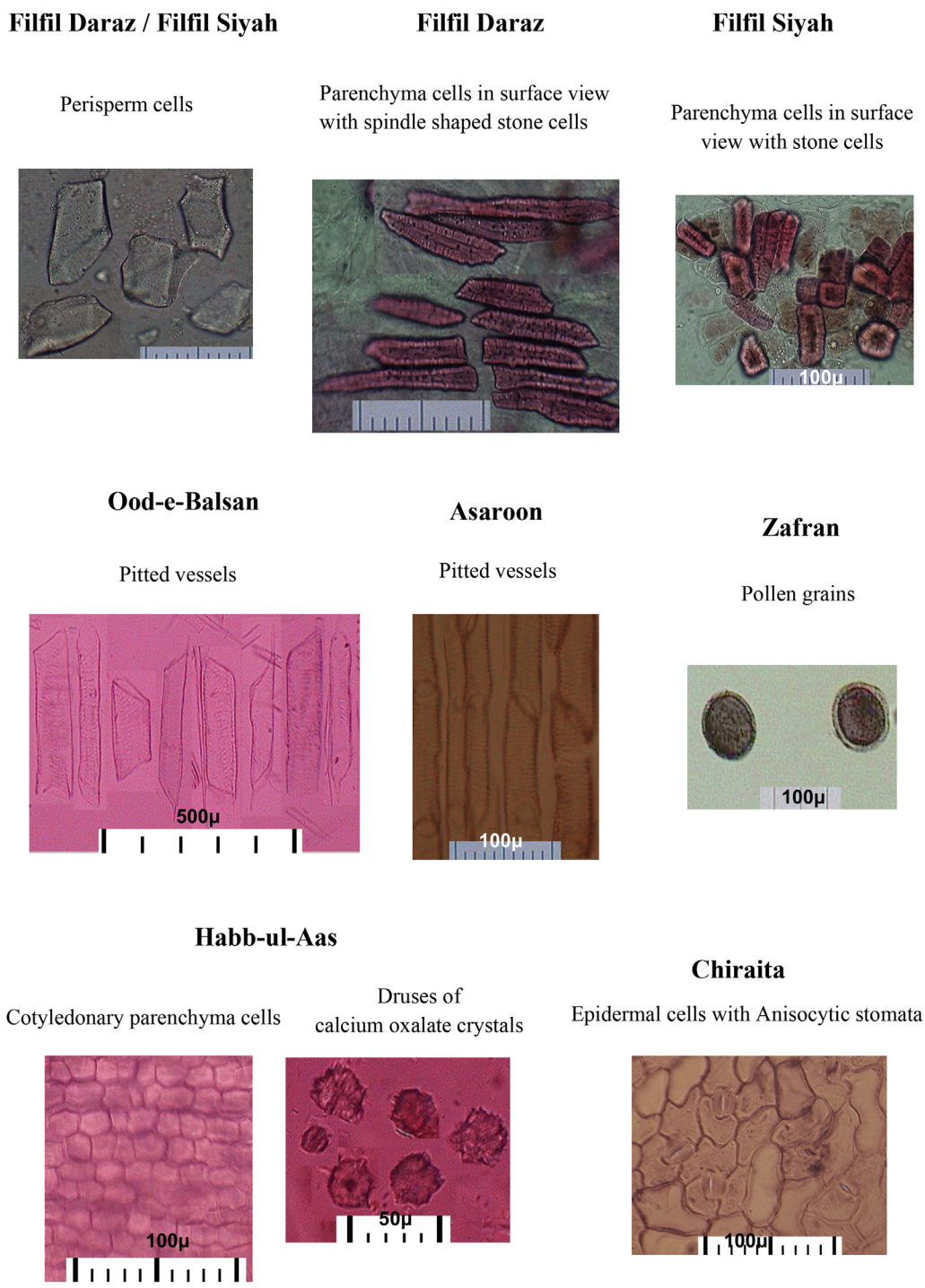

Fig. 1 – *(continued)*.

watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid, mounted in glycerin. The salient microscopic features of the drug were observed in different mounts.[4]

2.4. *Physico-chemical analysis*

All the three batch samples were subjected for the analysis of physico-chemical studies like total ash, acid insoluble ash, water soluble ash, solubility in alcohol and water and for loss on drying at 105 °C. Bulk density, sugar estimation and pH values for 1% and 10% aqueous solution were also carried out.[5]

2.5. *Thin layer chromatography*

2.5.1. *Preparation of extracts for TLC*

All the three samples (2 g) were soaked in chloroform and alcohol separately for 18 h, refluxed for 10 min on water bath and filtered. The filtrates were concentrated on water bath and made up to 5 ml in a standard flask separately.

2.5.2. *Method of developing for TLC*

Both chloroform and alcohol extracts were applied on pre-coated silica gel 60 F_{254} TLC plate (E. merck) as absorbent and developed the plate using solvent systems, toluene:ethyl acetate 9:1 and 6:4 respectively. After developing, the plates were dried and observed the colour spots at UV 254 nm, UV 366 nm and vanillin–sulphuric acid spraying reagent.[6]

2.6. *Quality control parameters*

The other parameters such as microbial load and heavy metal were carried out as per the WHO guidelines.[7] Aflatoxin and pesticide residues were carried out by standard methods.[8]

3. Results and discussion

Jawarish-e-Jalinoos is brown in colour, semi-solid, characteristic of its own odour and sweetish bitter in taste. The samples were spreaded in a petridish and observed. No filth, fungus or objectionable extraneous matters were found in the samples.

3.1. *Microscopical observation*

The salient features of raw drugs in Jawarish-e-Jalinoos were observed and the microscopical photographs are shown in Fig. 1

Vessels with scalariform thickening of length upto 150 μ and breath upto 50 μ **(Sumbul-ut-Teeb/Qust)**; group of bulbous perisperm cells packed with starch grains with tiny prismatic crystal of calcium oxalate, elongated thin walled parenchyma cells from aril tissue, orange coloured sclerenchyma cells in surface view **(Heel Khurd)**; pollen grains tetrahedral or spherical measuring upto 25 μ **(Qaranful)**; fibre like sclereids closely packed scalariform cells packed in regular rows of fairly uniform size upto 30 μ width **(Sad Kufi)**; non-lignified septate fibres upto 30 μ, vessels (non-lignified) with spiral, scalariform and reticulate thickenings upto 70 μ and fragments of reticulate vessels upto 100 μ **(Zanjabeel)**; numerous starch grains of various shapes and sizes **(Zanjabeel & Khulanjan)**; perisperm cells isolated or in groups with angular walls filled with aleurone grains and minute calcium oxalate crystals **(Filfil Siyah & Filfil Daraz)**; parenchyma cells in surface view with elongated or spindle shaped stone cells of length upto 150 μ and breadth upto 35 μ with a broad lumen upto 20 μ **(Filfil Daraz)**; stone cells polygonal upto 60 μ interspersed among parenchyma cells with circular lumen **(Filfil Siyah)**; vessels with pitted thickening of length upto 200 μ and breadth upto 50 μ with oblique end walls and simple perforation plate **(Asaroon)**; fibres thick walled lignified with striated walls and narrow lumen of length upto 1000 μ and breadth upto 40 μ and very large stone cells upto 200 μ, stone cells with horse shoe shaped thickenings upto 70 μ **(Saleekha/Darchini)**; cotyledonary parenchyma cells in surface view, druses of calcium oxalate crystals upto 40 μ **(Habb-ul-Aas)**; very few pollen grains spherical, nearly smooth in outline with clear exine and intine, size upto 120 μ **(Zafran)**; vessels with pitted thickening of length upto 500 μ and breadth upto 100 μ with oblique end walls and occasionally tailed **(Ood-e-Balsan)**; epidermal cells in surface view with anisocytic type of stomata **(Chiraita)**.

Table 2 – Physico-chemical parameters.

Parameters	Batch number					
	I	Mean value	II	Mean value	III	Mean value
Alcohol soluble matter (% W/W)	40.48 40.52 40.72	40.57	40.96 41.08 41.19	41.07	40.68 40.76 40.84	40.76
Water soluble matter (% W/W)	62.40 62.48 62.76	62.55	62.96 63.08 63.12	63.05	62.68 62.72 62.84	62.74
Total ash (% W/W)	0.79 0.82 0.86	0.82	0.80 0.84 0.88	0.84	0.78 0.83 0.87	0.83
Acid insoluble ash (%W/W)	0.16 0.18 0.21	0.18	0.19 0.22 0.24	0.22	0.17 0.20 0.27	0.21
pH values 1% Aqueous solution	5.63 5.81 5.90	5.78	5.65 5.72 5.97	5.78	5.58 5.69 5.77	5.68
pH values 10% Aqueous solution	4.13 4.27 4.46	4.29	4.05 4.27 4.31	4.21	4.29 4.37 4.42	4.36
Sugar estimation Reducing sugar (% W/W)	30.04 30.17 30.21	30.14	30.40 30.61 30.66	30.55	30.43 30.51 30.57	30.50
Non reducing sugar (% W/W)	6.36 6.44 6.68	6.49	6.08 6.14 6.17	6.13	6.28 6.31 6.41	6.33
Moisture (% W/W)	19.55 19.67 19.83	19.68	19.74 19.88 19.94	19.85	19.99 20.07 20.16	20.07
Bulk density	1.4835 1.4852 1.4889	1.4858	1.4779 1.4786 1.4795	1.4786	1.4805 1.4824 1.4864	1.4831

3.2. *Chemical analysis*

The moisture was obtained in the drug 19.87%. The alcohol soluble extractive (40.80%) might be due to the extraction of

Table 3 – R_f values of chloroform extract.

R_f values		
UV 254 nm	UV 366 nm	V. S. Reagent
0.95 Pink	0.95 Blue	0.96 Violet
0.80 Light pink	0.83 Light blue	0.78 Blue
0.68 Light pink	0.67 Light blue	0.69 Grey
0.60 Pink	0.56 Light blue	0.64 Violet
0.50 Light pink	0.41 Fluorescent blue	0.56 Brown
0.43 Light pink	0.28 Blue	0.53 Blue
0.34 Pink	0.21 Light blue	0.43 Violet
0.27 Yellowish green	0.17 Yellowish blue	0.35 Pink
0.15 Pink	0.12 Blue	0.24 Yellowish green
0.12 Pink		0.13 Violet

Table 4 – R_f values of alcohol extract.

R_f values		
UV 254 nm	UV 366 nm	V. S. Reagent
0.95 Pink	0.88 Light blue	0.96 Violet
0.69 Light pink	0.79 Blue	0.81 Brown
0.62 Pink	0.51 Blue	0.72 Green
0.50 Pink	0.43 Blue	0.68 Violet
0.44 Pink	0.20 Light blue	0.62 Blue
0.29 Pink		0.56 Grey
0.20 Light blue		0.50 Grey
		0.34 Pink
		0.20 Grey

polar chemicals constituents and the water soluble extractives 62.78% indicate the presence of inorganic constituents. The physico-chemical results are shown in Table 2.

3.3. *Thin layer chromatography analysis*

The chloroform and alcohol extract of all the three batch samples showed identical spots in UV 254 nm and 366 nm ranges and the R_f values of both the extracts are shown in Tables 3 and 4. The plates were developed using vanillin–sulphuric acid and heated at 105° till appears coloured spots in Figs. 2 and 3.

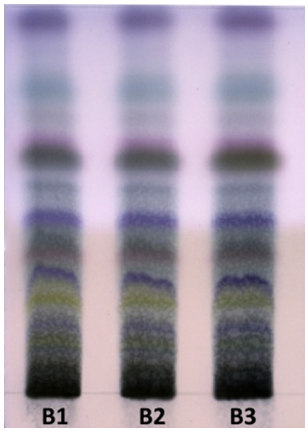

Fig. 2 – Solvent system, Toluene:Ethyl acetate (9:1): V. S. Reagent.

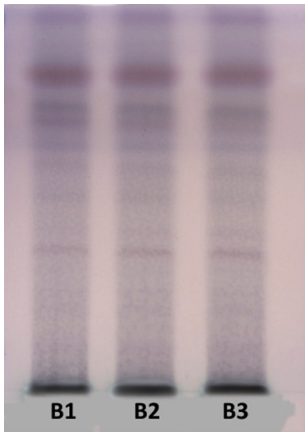

Fig. 3 – Solvent system, Toluene:Ethyl acetate (6:4): V. S. Reagent.

Table 5 – Analysis of microbial load.

S. no.	Parameter analysed	Results	WHO limits
1	Total Bacterial Count	4500 CFU/g	10^5 CFU/g
2	Total Fungal Count	Nil/g	10^3 CFU/g
3	Enterobacteriaceae	Absent/g	10^3 CFU/g
4	*Salmonella*	Absent/g	Nil
5	*Staphylococcus aureus*	Absent/g	Nil

Table 6 – Estimation of heavy metals.

S. no.	Parameter analysed	Results	WHO & FDA limits
1	Arsenic	0.0031	10 ppm
2	Cadmium	Nil	0.30 ppm
3	Lead	0.0025	10 ppm
4	Mercury	Nil	1.0 ppm

3.4. *Quality control parameters*

The WHO parameters such as microbial load and heavy metals were found within the permissible limit (Tables 5 and

Table 7 – Estimation of aflatoxins.

S. no.	Aflatoxins	Results
1	B_1	Nil
2	B_2	Nil
3	G_1	Nil
4	G_2	Nil

Table 8 – Analysis of pesticide residues.

S. no.	Pesticide residues	Results
1	Organo Chlorine Group	ND
2	Organo Phosphorus Group	ND
3	Acephate	ND
4	Chlordane	ND
5	Dimethoate	ND
6	Endosulphan	ND
7	Endosulfan	ND
8	Endosulfan	ND
9	Ethion	ND
10	Endosulfan sulphate	ND
11	Fenthion	ND
12	Heptachlor	ND
13	Lindane	ND
14	Methoxychlor	ND
15	Phorate sulfoxide	ND
16	Phorate sulfone	ND

ND – Not detected

6). The other parameters like aflatoxins B_1, B_2, G_1 and G_2 and pesticide residues – organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion were not detected from the drug samples (Tables 7 and 8).

Conflicts of interest

All authors have none to declare.

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