Standardization of Unani Drug – Jawarish Usquf

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Abstract

The drug Jawarish Usquf is therapeutically used in the ailments of Munaqqi-e-Asab, Laqwa and Qulanj. Jawarish Usquf was prepared in three different batches as per the guidelines of NFUM (Part-IV). Present study was aimed to evaluate the powder microscopical studies to identify the raw drugs present in the formulation, physico-chemical data to lay down pharmacopoeial standards, TLC to develop the fingerprints and WHO parameters to ascertain quality of the drug. Powder microscopical studies showed the presence of numerous starch grains, non-lignified septate fibres, reticulate vessels (Zanjabeel); fibres lignified not over 30µ breadth, stone cells horse shoe shaped (Darchini); large mesocarpic parenchyma cells with corner thickening (Aamla); pollen grains tetrahedral upto 20ì, fragments of anther wall (Qaranfal); tracheidal cells with scalariform thickening upto 50µ (Bisfayej); endosperm cells filled with starch grains and crystalloid proteins (Jauzbuwa); perisperm cells with angular walls filled with starch grains (Filfil Siyah); group of bulbous perisperm cells packed with starch grains (Heel Kalan); vessels with pitted thickening; rosette of calcium oxalate crystals (Turbud). The physico-chemical data showed that the drug contains moisture (18.51%), total ash (0.69%), acid in-soluble ash (0.022%) solubility in alcohol (25.46%) and water (64.55%). TLC study showed various spots at 254nm, 366nm and visible light (V-S reagent). The quality control study revealed the absence of microbial load, aflatoxins, heavy metal and pesticide residues. The evaluated standards will be much useful for laying down the pharmacopoeial standards of Jawarish Usquf and also in providing the quality medicine to the needy mass.

Keywords: Jawarish Usquf, Physico-chemical, TLC, and WHO parameters

Introduction

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Medicinal plants are the major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only 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. Standardization of herbal formulations is essential in order to assess the quality of drugs (Yadav & Dixit, 2008; Bandaranayake, 2006; Myers and Cheras, 2004).

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The microscopic characters of each ingredient used in the formulations are very difficult to identify and also some time there are overlapping with the character of other ingredient. Pharmacognostical study of a drug is an essential requirement to establish the presence of each ingredient in the formulations. 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 same formulation as well as variation among the same formulation procured from different sources.

The drug Jawarish Usquf (Anonymous, 2006) is one of the classical herbal Unani compound formulations. It is therapeutically useful in the ailments of Munaqqi-e-Asab (Nervine tonic), Laqwa (Facial paralysis) and Qulanj (Colic). The preparation of the drug Jawarish Usquf is based on traditional methods in accordance with the procedure given in NFUM, Part-IV (Anonymous, 2006) and it was prepared in three different batches.

The present study is an attempt to prepare and standardized the herbal formulation using pharmacopoeial studies such as organoleptic characters, microscopical, physico-chemical, TLC and quality control parameters.

Material and Methods

Ingredients Authentications

The raw drugs namely Zanjabeel, Darchini, Aamla Munaqqa, Qaranful, Bisfayej, Jauzbuwa, Filfil Siyah, Heel Kalan, Saqmonia, Turbud and Qand Safaid of the formulation were procured from raw drugs dealers of Chennai. These raw drugs were authenticated as per pharmacopoeial and other official standards (Anonymous, 2007 & 2008).

Drug Preparation

The drug was prepared in different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM, Part – IV (Anonymous, 2006) (Table 1). Take the required quantity of all the ingredients of pharmacopoeial quality. The raw drugs (1 to 2 and 4 to 10) were cleaned, dried, powdered and sieved through 80 mesh and kept separately. Aamla Munaqqa was grinded and made into paste and kept separately. Dissolve the specified quantity of sugar (11) in 375ml of water on slow heat. Add 0.1% citric acid and made the quiwam of 74% consistency then add 0.1% sodium benzoate, add the paste of aamla and mixed thoroughly. The quiwam was re-corrected to 77% consistency. The container was removed from fire while the quiwam is in hot add the mixed powders of ingredient number 1 to 2 and 4 to 10 and mixed thoroughly to prepare the

homogenous product. Allowed to cool to room temperature and packed it in tightly closed containers to protect from light and moisture.

Pharmacopoeial Standards

The pharmacopoeial studies such as organoleptic characters, microscopical, physic-chemical, TLC and quality control parameters were carried out:

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

ii. Powder microscopical studies: The drug sample (5g) was weighed and mixed with 50ml 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 glycerine. A few mg was taken in watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid, mounted in glycerine. The salient features of the drug were observed in different mounts (Wallis, 1997 and Johansen, 1940).

iii. Physico-chemical analysis: The moisture content at 105°C, ash values, solubility in water and alcohol, pH values, bulk density and sugar content etc., are the useful tools in standardisation of a herbal products as per standards method (Anonymous, 1987 and 1998).

iv. Thin layer chromatography: The drug samples (2g) were soaked in chloroform and alcohol separately for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made up to 5ml in a standard flask separately and carried out the TLC studies (Wagner *et al.*, 1984).

v. Other quality control parameter: The usage of herbal products along with higher safety margins, WHO has taken necessary step to ensure quality control parameters with the modern techniques and application of suitable standards. The microbial load and heavy metal parameters were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxins and pesticide residues were carried out by standard methods (Anonymous, 1997 and 2005).

Results and Discussion

Organoleptic characters: Jawarish Usquf is a brown semi-solid product with sweetish bitter in taste.

Microscopical observations: Isolated starch grains, simple oval to round shaped measuring upto 70ì, hilum eccentric, lamellae distinct; non-lignified septate fibres upto 50ì, reticulate vessels and fragments of reticulate vessels upto 70ì (Zanjabeel); fibres thick walled lignified with striated walls and narrow lumen of length upto 1000µ and breadth not over 30µ, stone cells with horse shoe shaped thickenings upto 100µ (Darchini); epidermal cells in surface view with uniformly thick walled cells containing silica crystals and with occasional paracytic stomata, mesocarpic parenchyma cells with large irregular thick walled cells showing corner thickening (Aamla); pollen grains tetrahedral spherical biconvex measuring upto 20ì in diameter, parenchyma cells with schizolysigenous oil glands, fragments of anther wall in surface view (Qaranfal); tracheidal cells with scalariform thickening upto 50µ (Bisfayej); endosperm cells in surface view with numerous starch grains and crystalloid proteins, each crystalloid proteins upto 40µ, perisperm cells in surface view filled with reddish brown contents (Jauzbuwa); perisperm cells isolated or in groups with angular walls filled with simple and compound starch grains and minute calcium oxalate crystals, stone cells polygonal upto 60µ interspersed among parenchyma cells with circular lumen (Filfil Siyah); group of bulbous perisperm cells packed with starch grains and tiny prismatic crystal of calcium oxalate, elongated thin walled parenchyma cells from aril tissue, orange coloured thick walled sclerenchyma cells in surface view (Heel Kalan); vessels with pitted thickening (simple pits) of length upto 400µ, breadth upto 200µ; rosette of calcium oxalate crystals upto 60µ, starch grains simple and compound; simple starch grains elliptical to spherical with central cleft hilum upto 25µ, compound starch grains 2 to 4 grains unite; medullary ray parenchyma cell filled with starch grains (Turbud) (Fig.1).

Physico-chemical: The physico-chemical analysis such as moisture content obtained in the drug was 18.51%. The alcohol soluble extractive (25.46%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (64.55%) 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.

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.

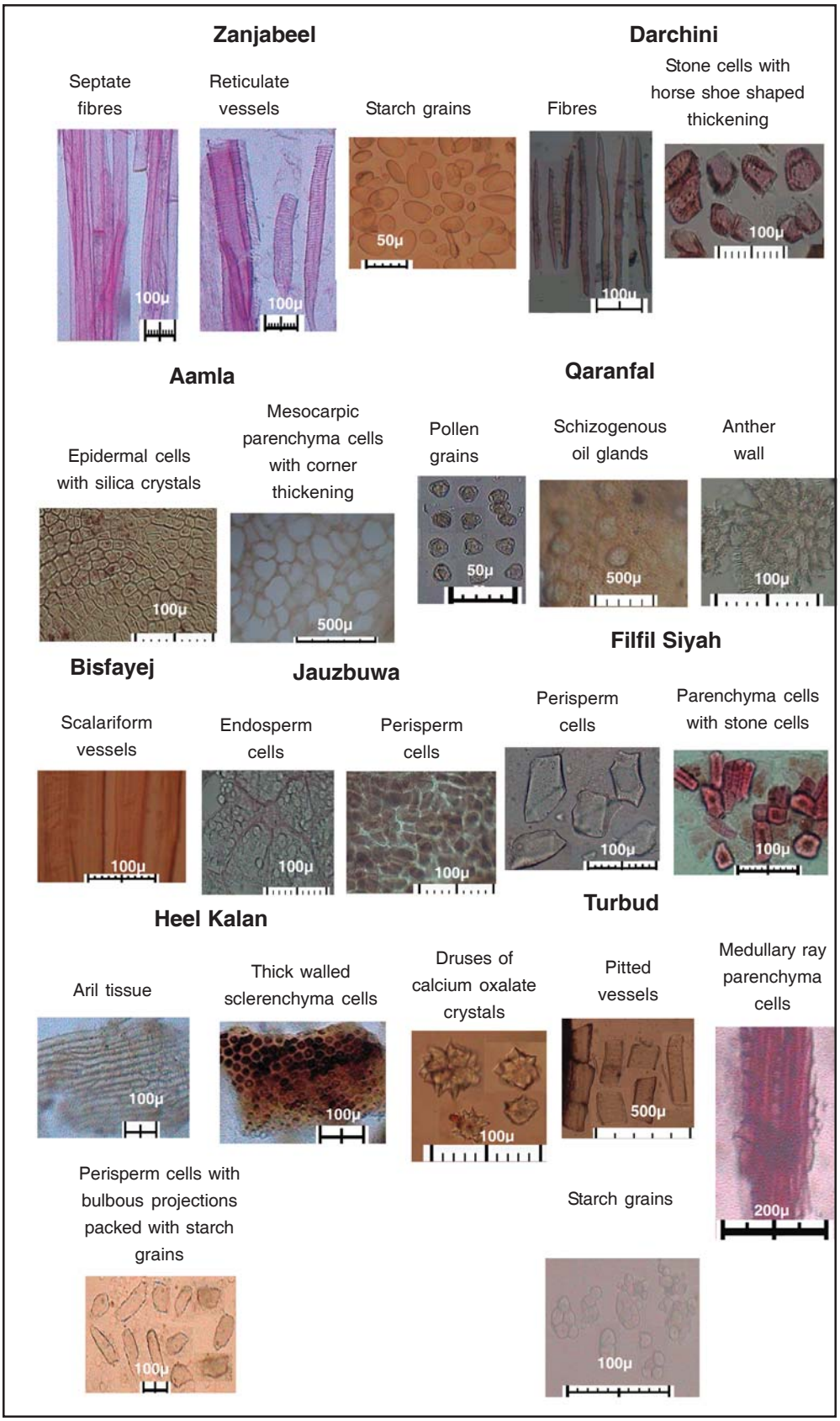


Fig. 1: Powder microscopy of Jawarish Usquf

Table 1: List of Ingredients in Jawarish Usquf

S.No.	Unani name	Unani Name	Part used	Quantity
1.	Zanjabeel Khushk UPI-I	*Zingiber officinale* Rosc	Rhizome	10 g.
2.	Darchini UPI-I	*Cinnamomum zeylanicum* Blume.	Inner stem bark	10 g.
3.	Aamla Munaqqa UPI-I	*Emblica officinalis* Gaertn.	Fruit	10 g.
4.	Qaranful UPI-I	*Syzygium aromaticum* (L.) Merr. L M Perry	Flower bud	10 g.
5.	Bisfayaj UPI-II	*Polypodium vulgare* Linn	Rhizome	10 g.
6.	Jauzbuwa UPI-I	*Myristica fragrans* Houtt.	Endosperm	10 g.
7.	Filfil Siyah UPI-IV	*Piper nigrum* Linn.	Fruit	15 g.
8.	Heel Kalan UPI-IV	*Ammomum subulatum* Roxb.	Fruit	15 g.
9.	Saqmonia	*Convolvulus scammonia* L.	Resin	15 g.
10.	Turbud UPI-V	*Operculina turpethum* Linn.	Root	15 g.
11.	Qand Safaid	Sugar	—	305 g.

Table 2: Physico-chemical parameters

Parameters	Batch Number		
	I	II	III
Extractives			
Alcohol soluble matter	25.21%; 25.40%; 25.52%	25.64%; 25.72%; 25.84%	25.18%; 25.24%; 25.36%
Water soluble matter	64.14%; 64.20%; 64.32%	64.44%; 64.56%; 64.76%	64.72%; 64.88%; 64.96%
Ash			
Total ash	0.71%; 0.74%; 0.81%	0.58%; 0.64%; 0.86%	0.54%; 0.59%; 0.77%
Acid insoluble ash	0.018%; 0.022%; 0.027%	0.013%; 0.017%; 0.025%	0.017%; 0.026%; 0.031%
pH values			
1% Aqueous solution	5.14; 5.23; 5.44	5.17; 5.31; 5.39	5.09; 5.24; 5.49
10% Aqueous solution	4.34; 4.39; 4.60	4.28; 4.31; 4.53	4.37; 4.42; 4.53
Sugar estimation			
Reducing sugar	24.22%; 22.32%; 22.39%	24.12%; 24.25%; 24.37%	24.17%; 24.27%; 24.33%
Non-reducing sugar	21.38%; 21.53%; 21.60%	21.57%; 21.70%; 21.81%	21.49%; 21.62%; 21.79%
Moisture	18.17%; 18.21%; 18.38%	18.35%; 18.44%; 18.58%	18.68%; 18.83%; 18.96%
Bulk Density	1.2805; 1.2875; 1.907	1.2799; 1.2898; 1.2954	1.2785; 1.2856; 1.2897

Table 3: *Rf* Values of chloroform extract

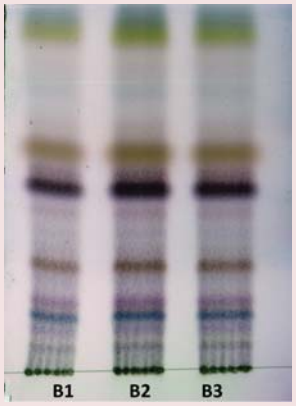
Solvent system Toluene: Ethyl acetate (9: 1)	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
	0.89 Brown	0.93 Brown	0.92 Yellowish green
	0.73 Pink	0.74 Fluorescent blue	0.82 Grey
	0.61 Pink	0.66 Fluorescent blue	0.73 Grey
	0.52 Pink	0.51 Light blue	0.58 Brown
	0.46 Pink	0.45 Blue	0.48 Violet
	0.41 Light pink	0.33 Blue	0.41 Grey
	0.28 Pink	0.20 Blue	0.26 Brown
	0.25 Light pink		0.18 Violet
	0.13 Pink		0.13 Pink
Fig. 2 V.S. Reagent			

Table 4: *Rf* Values of alcohol extract

Solvent system Toluene: Ethyl acetate (6: 4)	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
	0.93 Grey	0.92 Yellow	0.87 Violet
	0.83 Pink	0.85 Fluorescent blue	0.85 Brown
	0.66 Light pink	0.79 Blue	0.81 Violet
	0.59 Light pink	0.72 Blue	0.71 Grey
	0.55 Pink	0.67 Blue	0.64 Violet
	0.49 Light pink	0.56 Light blue	0.56 Yellowish green
	0.29 Light pink	0.49 Blue	0.32 Grey
	0.18 Light pink	0.19 Light blue	0.31 Light grey
Fig.- 3 V. S. Reagent			

Table 5: Analysis of Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	200 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	200 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	Not detected	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

Conclusion

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

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