Quality Evaluation of Jawarish-e-Ood Kibreet

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Abstract

Herbal products have become a major part in curing the different kinds of human ailments. In India, the Unani system of medicine consists of large number of herbal products to cure the different type of diseases. Jawarish-e-Ood Kibreet is a Unani herbal formulation prepared in combination of ingredients like Ood, Dana-e-Heel Khurd, Dana Heel Kalan, Sazaj Hindi, Post-e-Turanj, Tabasheer, Nana Khushk, Darchini, Gul-e-Surkh, Zarambad, Qaranful, Jauzbuwa, Bisbasa, Nabat Safaid and Arq-e-Kibreet. It is prescribed in the treatment of Zof-e-Meda (weakness of the stomach) and Zof-e-Ishteha (anorexia) disorders. Evaluation of Unani formulations has become a fundamental requirement of the research organizations. Based on the available sources, an attempt has been made to evaluate the drug Jawarish-e-Ood Kibreet. To evaluate the drug the parameters like macroscopic, microscopic, determination of moisture content, ash values, bulk density, pH values, extractive values, TLC and analysis of quality control parameters viz. heavy metals, microbial content, aflatoxins and pesticide residues were performed. The evaluated data shall help to lay down pharmacopoeial standards for the drug Jawarish-e-Ood Kibreet and also in producing quality and efficacious products having batch to batch consistency.

Keywords: Jawarish-e-Ood Kibreet, Powder microscopy, Physico-chemical, TLC and WHO parameters

Introduction

Jawarish-e-Ood Kibreet (Anonymous, 2006) is one of the ancient commonly used Unani formulations. The poly herbal formulation is prepared using 15 ingredients (Table 1). The drug is prescribed for the treatment of Zof-e-Meda (Weakness of the stomach) and Zof-e-Ishteha (Anorexia) disorders. Evaluation of Unani medicines with the perspective of safety, efficacy and quality will not only preserve the traditional heritage but also rationalize their uses of Unani medicines in the health care.

Due to lack of standards and quality control methods there are batch to batch variations in the similar formulations. The main requirement of a standardisation is to establish the presence of each ingredient in the formulations (Bandaranayake, 2006; Myers and Cheras, 2004). The present study is an attempt to evaluate the drug by applying modern parameters such as microscopical, physico-chemical, thin layer chromatography and WHO parameters viz., microbial load, aflatoxin, heavy metals and pesticide residue.

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Table 1: List of the raw drugs of Jawarish-e-Ood Kibreet Formulation

S. No.	Unani name	Botanical/ English name	Part used	Quantity taken for SOP
1.	Ood	*Aquilaria agallocha* Roxb.	Heart wood	20 g.
2.	Dana-e-Heel Khurd	*Elettaria cardamomum* (L) Maton.	Seed	6 g.
3.	Dana Heel Kalan	*Amomum subulatum* Roxb.	Seed	6 g.
4.	Sazaj Hindi	*Cinnamomum tamala* (Buch. Ham.) Nees & Eberm.	Leaves	6 g.
5.	Post-e-Turanj	*Citrus medica* Linn.	Pericarp	6 g.
6.	Tabasheer	*Bambusa bambos* Druce.	Bamboo Manna	6 g.
7.	Nana	*Mentha viridis* Linn.	Aerial part	6 g.
8.	Darchini	*Cinnamomum zeylanicum* Blume.	Inner stem bark	6 g.
9.	Gul-e-Surkh	*Rosa damascena* Mill.	Flower	10 g.
10.	Zarambad	*Curcuma zeodaria* Li	Rhizome	4 g.
11.	Qaranful	*Syzygium aromaticum* (L.) Merr. L M Perry.	—	4 g.
12.	Jauzbuwa	*Myristica fragrans* Houtt.	Endosperm	4 g.
13.	Bisbasa	*Myristica fragrans* Houtt.	Aril	4 g.
14.	Nabat Safaid	Sugar	Crystals	350 g.
15.	Arq-e-Kibreet	Sulphur extract	Extract	600 ml.

Material and Methods

Collection of the raw drugs

Genuine raw drugs namely Ood, Dana Heel Khurd, Dana Heel Kalan, Sazaj Hindi, Post-e-Turanj, Tabasheer, Nana, Darchini, Gul-e-Surkh, Zarambad, Qaranful, Jauzbuwa, Bisbasa, Nabat Safaid and Arq-e-Kibreet of the formulation were procured from raw drugs dealers of Chennai. The raw drugs were identified using pharmacognostical methods and evaluated their pharmacopoeial standards.

Preparation of the drug

The ploy herbal semisolid drug was prepared in different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM, Part–IV (Table I).

Powder microscopy

Microscopical examination allows more detail of a drug and can be used to identify the organised drugs by their known histological characters viz., cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibres and vessels (Kokate *et al*., 2000).

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. Then few mg was taken in watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid, mounted in glycerine. The microscopic salient features of the drug were observed in different mounts (Wallis, 1997 & Johansen, 1940).

Physico-chemical analysis

The physico-chemical methods viz., moisture content, ash values, solubility in different solvents, pH values, bulk density and sugar content etc., are useful tools in standardisation of a herbal product for maintaining batch to batch consistency. The drug samples were subjected for the standardisation of physico-chemical parameters and analysed as per the standards method (Anonymous, 1987).

Thin layer chromatography

Thin Layer Chromatography is a physical method of separation in which the components to be separated are distributed between two phases; one of these is a stationary phase bed and other is a mobile phase which percolates through this bed. It offers the best method for recording the finger prints which can be reproduced anywhere at the same laboratory condition of a particular product.

The samples of the drug (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).

Quality control parameters

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 parameters such as microbial load and heavy metal were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxin and pesticide residues were carried out by standard methods (Anonymous, 2000).

Results and Discussion

Jawarish-e-Ood Kibreet is a dark brown semi-solid product with sweetish bitter in taste.

Microscopical observation

The salient features of the raw drugs used in the preparation of Jawarish-e-Ood Kibreet were evaluated using powder microscopic studies and the photographs are shown in Fig. 1. Pitted vessels with simple perforation plate upto 200µ, xylem parenchyma lignified with pitted walls, xylem ray parenchyma cells along with fibres, septate fibres (Ood); perisperm cells isolated or in groups with bulbous projections filled with starch grains and tiny prismatic crystal of calcium oxalate, elongated thin walled parenchyma cells from aril tissue, thick walled sclerenchyma cells in surface view (Dana Heel Khurd / Dana Heel Kalan); epidermal cells in surface view with sunken stomata (paracytic stomata) subsidiary cells not clear (Sazaj Hindi); epidermal cells in surface view with circular stomata and schizolysigenous oil glands (Post-e-Turanj); numerous, angular, sharp edged siliceous sandy particles (Tabasheer); epidermal cells (bigger cells) in surface view with wavy margin, diacytic stomata, prominent capitate glandular trichomes upto 80µ in length with single basal cell and single head cell, labiaceous glandular trichomes with single basal cell and a head of 8 cells upto 80µ in diameter (Nana Khushk); 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); long simple unicellular covering trichome, pollen grains round to oval upto 35µ with three distinct germ pores (Gul-e-Surkh); numerous starch grains of various shapes and size upto 50µ (Zarambad); pollen grains tetrahedral spherical biconvex measuring upto 20ì in diameter, spindle shaped fibres, parenchyma cells with schizolysigenous oil glands, fragments of anther wall in surface view (Qaranfal); 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 content (Jouzbuwa); thick walled elongated parenchyma cells in surface view upto 50µ wide (Bisbasa).

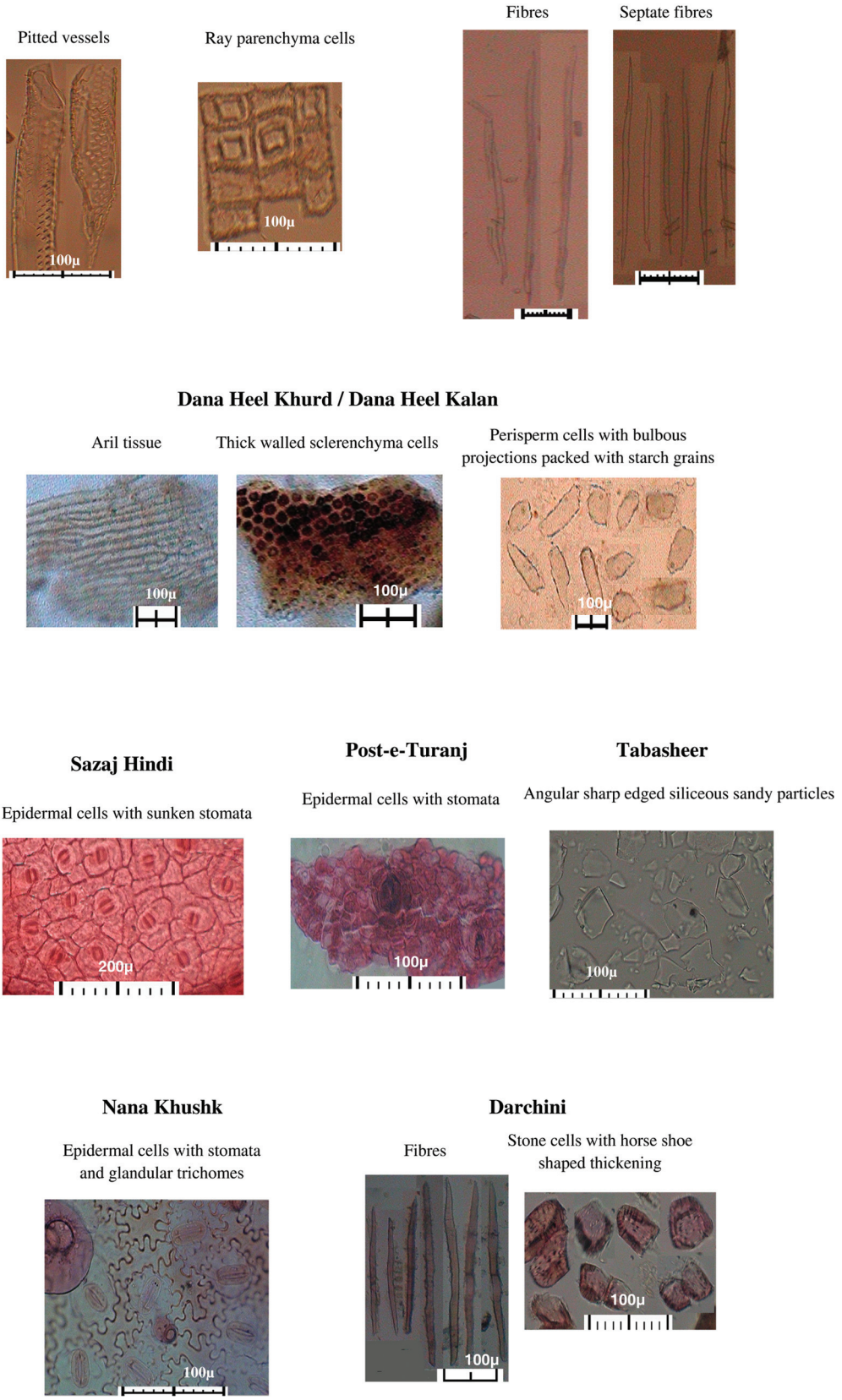


Fig. 1: Powder microscopy of Jawarish-e-Ood Kibreet

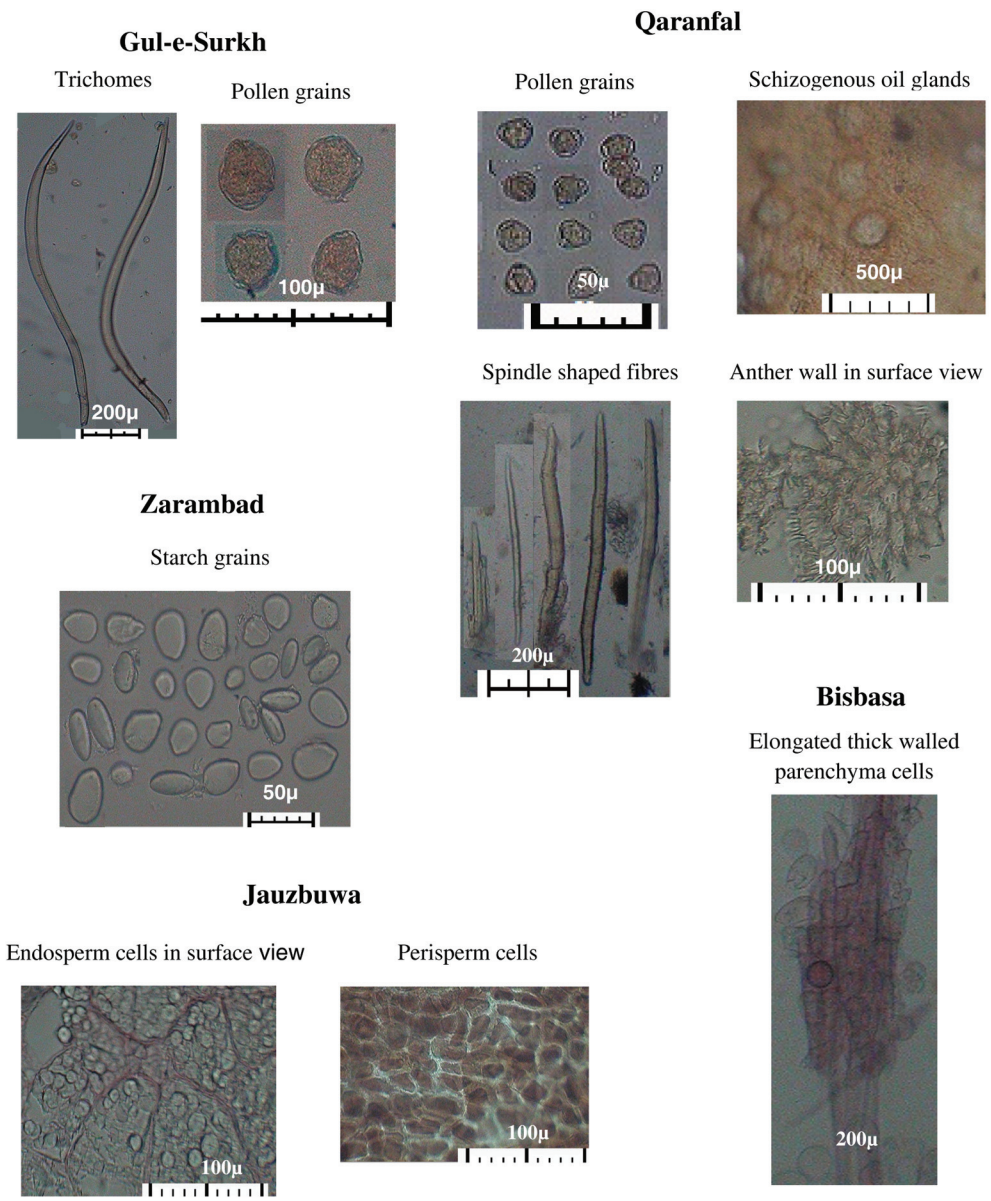


Fig. 1: *Continued...*

Chemical analysis

The physico-chemical data, moisture content was obtained in the drug 18.58%. The alcohol soluble extractive (58.50%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (63.49%) indicate the presence of inorganic constituents. The obtained data are shown in Table - 2.

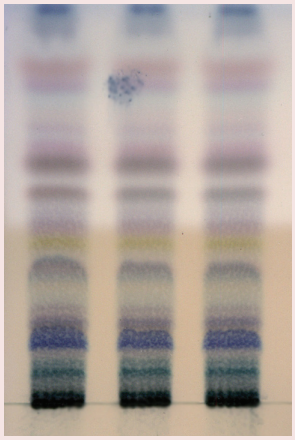
Thin Layer Chromatography analysis

The chloroform and alcohol extract of all the three batch samples showed identical spots at UV – 254nm and 366nm ranges and the R_f values of both the extracts

Table 2: Physico-chemical parameters

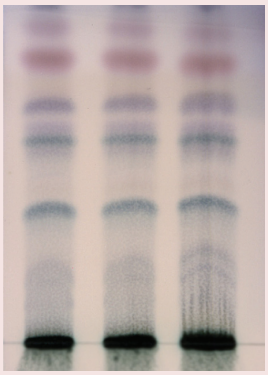
Parameters Analyzed	Batch Number (n=3)		
	I	II	III
Extractives Alcohol soluble matter Water soluble matter	58.52% 63.16%	58.24% 63.48%	58.76% 63.84%
Ash Total ash Acid insoluble ash	1.82% 0.95%	1.79% 0.89%	1.86% 0.98%
pH values 1% Aqueous solution 10% Aqueous solution	5.59 4.34	5.74 4.41	5.41 4.42
Sugar estimation Reducing sugar Non-reducing sugar	35.83% 7.55%	35.61% 7.74%	35.84% 7.53%
Moisture	18.81%	18.26%	18.68%
Bulk Density	1.3997	1.4087	1.4009

Table 3: *Rf* Values of chloroform extract

Solvent system Toluene: Ethyl acetate (9 : 1)	Rf Values		
	UV 254nm	UV 366nm	V. S. Reagent
 B1 B2 B3 Fig. 2 V. S. Reagent	0.93 Pink 0.71 Light pink 0.64 Pink 0.56 Light pink 0.52 Pink 0.46 Pink 0.32 Yellowish green 0.14 Light pink	0.90 Light blue 0.72 Brown 0.55 Blue 0.51 Red 0.42 Fluorescent blue 0.34 Red 0.14 Blue	0.94 Blue 0.82 Red 0.77 Grey 0.67 Grey 0.59 Brown 0.50 Brown 0.42 Grey 0.32 Violet 0.22 Light grey 0.19 Violet

are shown in Table 3 and 4. The plates were developed using vanillin-sulphuric acid and heated at 105ºC till appeared coloured spots shown in Fig. 2 and 3.

Table 4: *Rf* Values of alcohol extract

Solvent system Toluene: Ethyl acetate (6 : 4)	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
 B1 B2 B3 Fig. 2 V. S. Reagent	0.93 Yellowish green	0.93 Red	0.90 Brown
	0.81 Pink	0.81 Blue	0.81 Brown
	0.73 Light pink	0.78 Violet	0.69 Violet
	0.60 Pink	0.56 Yellow	0.59 Green
	0.57 Yellowish green	0.52 Blue	0.53 Light grey
	0.47 Light pink	0.47 Brown	0.39 Blue
	0.39 Light pink	0.40 Blue	0.26 Grey
	0.27 Yellow	0.32 Fluorescent blue	0.14 Grey
	0.15 Yellowish green	0.28 Red	
		0.17 Red	

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 - organo chlorine group, organo phosphorus group, acephate, chlordane, dimethoate, endosulphan, endosulfan, endosulfon, ethion, endosufon sulphate, fenthion, heptachlor, lindane, methoxychlor, phorate sulfoxide and phorate sulfone were not detected from the drug samples shown in Table 7 and 8.

Table 5: Analysis of Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	2,000 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	< 10 CFU/ gm	10^3 CFU / gm
3	Enterobacteriaceae	Absent / gm	10^3 CFU / gm
4	Salmonella	Absent / gm	Nil
5	*Staphylococcus aureus*	Absent / gm	Nil

Table 6: Estimation of Heavy Metals

S.No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Arsenic	Not detected	3 ppm
2	Cadmium	Not detected	0.3 ppm
3	Lead	0.0023	10 ppm
4	Mercury	Not detected	1.0 ppm

Table 7: Estimation of Aflatoxins

S.No.	Aflatoxins	Results	WHO Limits
1	B_1	ND	0. 5ppb
2	B_2	ND	0.1ppb
3	G_1	ND	0. 5ppb
4	G_2	ND	0.1ppb

ND = Not Detected

Table 8: Analysis of Pesticide Residues

S.No.	Pesticide Residues	Results	Limits
1	Organo Chlorine group	ND	(DL 0.005mg/Kg)
2	Organo Phosphorus group	ND	(DL 0.005mg/Kg)
3	Acephate	ND	(DL 0.005mg/Kg)
4	Chlordane	ND	(DL 0.005mg/Kg)
5	Dimethoate	ND	(DL 0.005mg/Kg)
6	Endosulphan	ND	(DL 0.005mg/Kg)
7	Endosulfan	ND	(DL 0.005mg/Kg)
8	Endosulfon	ND	(DL 0.005mg/Kg)
9	Ethion	ND	(DL 0.005mg/Kg)
10	Endosufon sulphate	ND	(DL 0.005mg/Kg)
11	Fenthion	ND	(DL 0.005mg/Kg)
12	Heptachlor	ND	(DL 0.005mg/Kg)
13	Lindane	ND	(DL 0.005mg/Kg)
14	Methoxychlor	ND	(DL 0.005mg/Kg)
15	Phorate sulfoxide	ND	(DL 0.005mg/Kg)
16	Phorate sulfone	ND	(DL 0.005mg/Kg)

ND – Not detected

Conclusion

The evaluated data such as powder microscopy, physico-chemical, TLC and quality control shows that the genuine raw drugs were added in the formulation and there is no variation in the batch to batch consistency of the drug.

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