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SCIENTIFIC VALIDATION OF JAWARISH-E-BISBASA - A UNANI FORMULATION

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ABSTRACT

The compound drug Jawarish-e-Bisbasa is a polyherbal Unani formulation. It is used in the ailments of general weakness of the stomach, flatulence in the stomach, indigestion, blind piles and nausea etc. Therefore the scientific validation for development of SOP, pharmacopoeial standards, TLC studies and safety parameter is very much essential to provide the quality medicine to the needy mass. The drug was prepared in different batches at laboratory scale using authenticated nine raw drugs. The present study was aimed to develop the SOP's for the preparation of drug and to evaluate its pharmacopoeial parameters, estimation of heavy metals, microbial load, aflatoxins and pesticide residues. The obtained physicochemical data will help in laying down SOP's and pharmacopoeial standards for this drug. Thin Layer Chromatographic studies of the drug will provide the finger prints which will help in quality control and detection of adulteration. The result of quality control parameters such as microbial load, aflatoxins, heavy metals and pesticide residue shows that the drug is free from toxic substances.

INTRODUCTION: Herbal drugs constitute a major part in all the traditional system of medicine [1] and there has been an increasing realization that TSM products are safer and this has lead to the spurt in the use of these plant based products in India.

Jawarish-e-Bisbasa is one of the polyherbal Unani compound formulations available in semisolid form in the market. The herbal formulation is being prescribed by Unani Physician for the treatment of weakness of the stomach, flatulence in the stomach, indigestion, blind piles and nausea [2].

The scientific standards like development of SOP's which includes procurement of ingredients, authentication, removal of adulteration if any, powdering the raw drug up to the required fineness, method of preparation, evaluation of physicochemical data and quality control parameters are essential to provide the quality medicine to the needy mass. The present study was designed to scientifically validate the drug by applying modern parameters such as microscopical, physicochemical, thin layer chromatography, microbial load, aflatoxin, heavy metal and pesticide residue.

MATERIALS AND METHODS: The raw drugs of the formulation were procured from local raw drugs dealers, Chennai with the knowledge of Unani Physician. The raw drugs were identified using pharmacognostical methods[3] and evaluated their pharmacopoeial standards. The drug Jawarish-e-Bisbasa was prepared in different batches at laboratory scale as per the formulation composition.

Composition of formulation: Jawarish-e-Bisbasa is a semi-solid preparation made with the following ingredients in the composition as given in **Table 1.**

TABLE 1: LIST OF INGREDIENTS OF THE JAWARISH-E-BISBASA FORMULATION

S. No.	Unani name	Botanical/ English name	Part used	Quantity taken for SOP
1.	Heel Kalan	*Ammomum subulatum* Roxb.	Fruit	50 g
2.	Bisbasa	*Myristica fragrans* Houtt.	Arillus	30 g
3.	Saleekha	*Cinnamomum cassia* Blume.	Stem bark	30 g
4.	Heel Khurd	*Elettaria cardamomum* (L) Maton	Fruit	30 g
5.	Zanjabeel	*Zingiber officinale* Rosc.	Rhizome	30 g
6.	Darchini	*Cinnamomum zeylanicum* Blume.	Stem bark	30 g
7.	Asaroon	*Asarum europaeum* Linn.	Rhizome	30 g
8.	Filfil Siyah	*Piper nigrum* Linn.	Fruit	20 g
9.	Qaranful	*Syzygium aromaticum* (L.) Merr. L M Perry	Flower bud	15 g
10.	Nabat Safaid	Sugar crystal		200 g
11.	Qand Safaid	Sugar	-	800 g

Method of Preparation: All the ingredients were taken of pharmacopoeial quality. Clean, dried and made the powders of the ingredients number 1 to 9 and sieved through 80 mesh and kept separately. Dissolved the specified quantity of ingredient number 10 and 11 in 1000 ml of water on slow heat and boiled the content, at the boiling stage 0.1% citric acid was added, mixed well and boiled to prepare the 76% consistency of Quiwam. Then the vessel was removed from the fire. While hot condition, the mixed powders of ingredients 1 to 9 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.

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

Chemical analysis: Prepared minimum three batch samples were subjected for chemical analysis. Physicochemical studies like total ash, acid insoluble ash, water soluble ash, alcohol and water solubility, loss on drying at 105^{o}C, microbial load and heavy metal were carried out as per the WHO guidelines [5]. Aflatoxin, pesticide residues were carried out by standard methods [6]. The bulk density, sugar estimation and *pH* values for 1% and 10% aqueous solution were also carried out [7].

Thin layer chromatography:

- **Preparation of extracts for TLC:** 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.
- **Method of developing for TLC:** Chloroform and alcohol extracts were applied on precoated silica gel 60 F_{254} TLC plate (E. Merck) as absorbent and developed the plate using solvent systems, toluene: ethyl acetate 9:1 and 1: 1 respectively. After developing, the plates were dried and observed the color spots at UV-254, UV-366nm and vanillin-sulphuric acid spraying reagent[8].

RESULTS AND DISCUSSION: Jawarish-e-Bisbasa is blackish brown in color, semi-solid, characteristic of its own odor and sweetish bitter in taste. Drug samples were spreaded in a petridish and observed. The samples did not show any filth, fungus or objectionable extraneous matter.

Microscopical Observation: The salient features of raw drugs used in Jawarish-e-Bisbasa are observed and the microscopical diagrams are shown in **Fig. 1**.

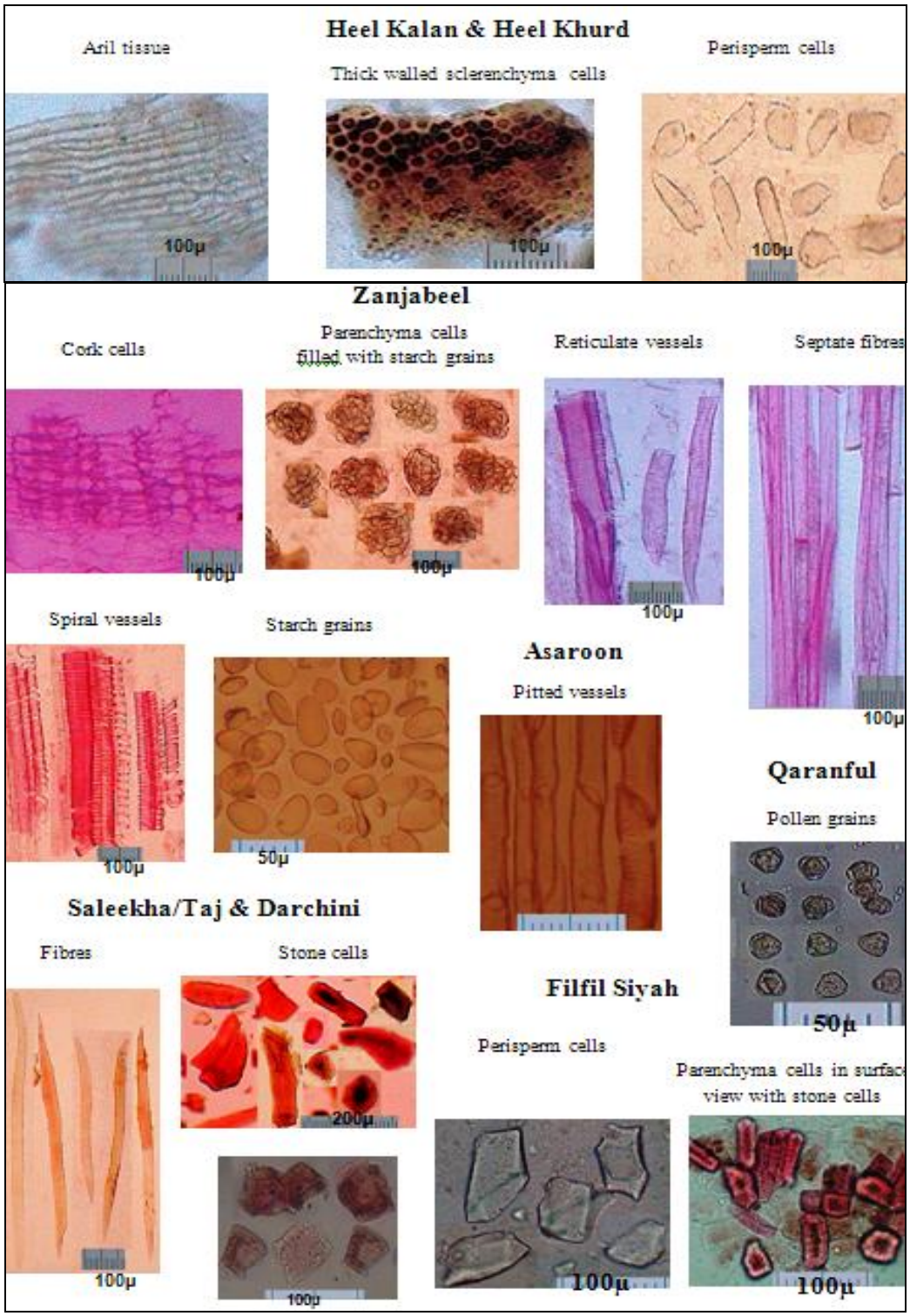

FIG. 1: POWDER MICROSCOPY OF JAWARISH-E-BISBASA

Perisperm cells with bulbous projections packed with starch grains and a few tiny prismatic crystal of calcium oxalate; fragments of aril tissue from testa; orange coloured sclerenchyma cells in surface view **(Heel Kalan & Heel Khurd);** large stone cells upto 300µ, stone cells with horse shoe shaped thickenings upto 100µ, numerous fibres with thick walled and very narrow lumen **(Saleekha/Taj & Darchini)**; stone cells polygonal upto 75µ interspersed among parenchyma cells with circular lumen, perisperm cells with angular walls isolated or in groups filled with starch grains **(Filfil Siyah)**; isolated starch grains, simple oval to round shaped measuring upto 70µ, hilum eccentric, lamellae distinct; non-lignified septate fibres upto 50µ, reticulate vessels and fragments of reticulate vessels upto 70µ; spiral vessels upto 70µ; cork cells in surface

view; parenchyma cells filled with abundant starch grains (**Zanjabeel**); vessels with pitted thickening of length upto 200µ and breadth upto 35µ with oblique end walls and simple perforation plate **(Asaroon)**; pollen grains tetrahedral, spherical, biconvex measuring upto 20µ **(Qaranful).**

Chemical analysis: Moisture content of the drug shows 17.75%. Alcohol soluble extractive (58.38%) might be due to the extraction of polar chemicals constituents and the water soluble extractives 63.88% indicate the presence of inorganic constituents. The physico-chemical data of the drug are shown in **Table 4**. The microbial load is found within the permissible limit **Table 5**. The heavy metals such as lead, cadmium, arsenic and mercury are present within the permissible limit **Table 6.** The studies on other parameters, aflatoxins such as B_1, B_2, G_1 and G_2 and pesticide residues like organo-chlorine group, organo-phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion shows that they are not detected in the drug samples **Table 7 & 8**.

Thin Layer Chromatography Analysis: Thin layer chromatography studies of chloroform and alcohol extract of all the three batch samples showed identical spots in various detector ranges. The R_f values of the chloroform and alcohol extracts are shown in **Table 2 and 3.** The plates were developed using vanillin-sulphuric acid and heated at 105º till appears colored spots in **Fig. 2 and 3.**

FIG. 2: THIN LAYER CHROMATOGRAPHY FOR CHLOROFORM EXTRACT

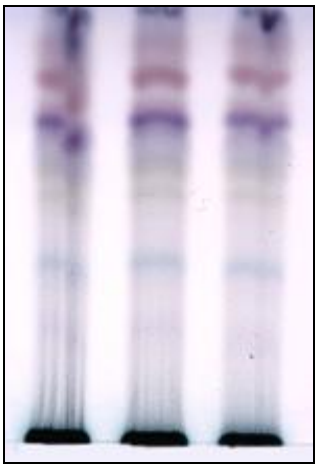

FIG. 3: THIN LAYER CHROMATOGRAPHY FOR ETHANOL EXTRACT
Detector: V. S. Reagent, Track 1: Batch – I, Track 2: Batch – II, Track 3: Batch – III

TABLE 2: Rf VALUES OF CHLOROFORM EXTRACT

Solvent system	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
Toluene: Ethyl acetate (9 : 1)	0.90 Light pink	0.93 Light blue	0.93 Grey
	0.82 Light pink	0.54 Blue	0.84 Pink
	0.78 Pink	0.40 Fluorescent blue	0.78 Violet
	0.66 Pink	0.28 Violet	0.66 Light blue
	0.57 Light pink	0.16 Yellow	0.58 Brown
	0.49 Light pink	0.12 Yellow	0.50 Pink
	0.40 Pink		0.44 Blue
	0.28 Pink		0.40 Violet
	0.21 Pink		0.37 Brown
	0.13 Pink		0.29 Pink
			0.25 Blue
			0.22 Pink
			0.18 Pink
			0.13 Blue

TABLE 3: Rf VALUES OF ALCOHOL EXTRACT

Solvent system	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
Toluene : Ethyl acetate (1 : 1)	0.96 Light pink 0.88 Light pink 0.81 Pink 0.72 Light pink 0.60 Pink 0.52 Pink 0.33 Light pink	0.85 Fluorescent blue 0.64 Violet 0.54 Light blue 0.42 Light blue 0.36 Light blue	0.97 Grey 0.93 Pink 0.82 Brown 0.72 Violet 0.62 Blue 0.54 Yellow 0.40 Blue 0.24 Grey

TABLE 4: PHYSICOCHEMICAL PARAMETERS

Parameters	Batch Number					
	I	**Mean value**	**II**	**Mean value**	**III**	**Mean value**
Alcohol soluble matter (% W/W)	58.28 58.36 58.44	58.36	58.72 58.80 58.92	58.81	57.88 57.96 58.08	57.97
Water soluble matter (% W/W)	63.56 63.60 63.72	63.62	63.84 63.88 63.96	63.89	64.08 64.12 64.20	64.13
Total ash (% W/W)	0.81 0.83 0.88	0.84	0.91 0.95 0.98	0.94	0.78 0.82 0.85	0.81
Acid insoluble ash (%W/W)	0.15 0.18 0.20	0.17	0.22 0.25 0.28	0.25	0.12 0.17 0.19	0.16
pH values 1% Aqueous solution	5.52 5.57 5.61	5.56	5.49 5.51 5.65	5.55	5.59 5.63 5.68	5.63
pH values 10% Aqueous solution	4.72 4.76 4.83	4.77	4.55 4.60 4.65	4.60	4.42 4.50 4.57	4.49
Sugar estimation Reducing sugar (% W/W)	43.45 43.49 43.55	43.49	43.47 43.52 43.58	43.52	43.60 43.76 43.82	43.72
Non reducing sugar (% W/W)	3.42 3.51 3.65	3.52	3.60 3.72 3.81	3.71	3.97 4.09 4.18	4.08
Moisture (% W/W)	17.23 17.38 17.45	17.35	17.68 17.74 17.80	17.74	18.09 18.18 18.28	18.17
Bulk Density	1.3788 1.3809 1.3856	1.3817	1.3994 1.4009 1.4058	1.4020	1.3796 1.3895 1.3987	1.3892

TABLE 5: ANALYSIS OF MICROBIAL LOAD

S. No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	3,000 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	Nil/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	Nil	10 ppm
2	Cadmium	Nil	0.30 ppm
3	Lead	Nil	10 ppm
4	Mercury	Nil	1.0 ppm

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	Endosulfon	ND
9	Ethion	ND
10	Endosufon 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	

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