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"EVALUATION OF PHARMACOPOEIAL STANDARDS WITH HPTLC PROFILE OF AN IMPORTANT UNANI FORMULATION - QURS-E-GULNAR"

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ABSTRACT

The preparation of classical Unani formulations is based on traditional methods in accordance with the procedures described in classical texts. Lack of modern pharmacopoeial standards for preparation of these formulations results in batch to batch variations and low quality finished products. The increase in the demand for herbal drugs has led to the need for quality standardization of these drugs. The Drug Standardisation Research Unit under the Central Council of Research in Unani medicine, New Delhi is engaged in developing the standards of classical Unani formulations. The Unani polyherbal formulation, Qurs-e-Gulnar known to be effective in the treatment of chronic diarrhoea, has been standardized at the Unit by following modern scientific quality control procedures. The formulation was subjected to various quality control parameters such as organoleptic evaluations (color, odor, taste, and consistency), physicochemical evaluations (loss on drying, total ash, acid insoluble ash, pH of 1 and 10% solution, water soluble matter and alcohol-soluble matter) and HPTLC analysis. The evaluation of contaminants such as heavy metals, aflatoxins, pesticide residues, and microbial contamination was also carried out in the formulation.

KEYWORDS: SOP, Microscopy, pharmacopoeial parameters, HPTLC, Qurs-e-Gulnar, Quality control.

INTRODUCTION

Qurs-e-Gulnar is a Unani poly-herbal formulation categorized under Aqras (tablets) in the National Formulary of Unani Medicine, Part -1.[1] Qurs-e-Gulnar is said to possess Habis (haemostatic) and Qabiz (astringent) action and is used in the treatment of Is'hal-e-Muzmin (chronic diarrhea), Is'hal-e-Damwi (bloody diarrhea) and Nafs-ud-Dam (haemoptysis) (Kabeeruddin). These properties can be attributed to the presence of ingredients like Gulnar (*Punica granatum* L.), Gil-e-Armani (Aluminium silicate), Samagh-e-Arabi(*Acacia nilotica* L. Wild ex. Del), Gul-e-Surkh(*Rosa damascena* Mill), Aqaqia (*Acacia nilotica* L. Wild ex. Del) and Kateera (*Cochlospermum religosum* (L) Alston).

Gulnar, the sterile flowers of *Punica granatum*, are used in Unani medicine for treatment of dysentery, hematemesis, epistaxis etc.[2] Gil-e-Armani is a mineral origin drug used in Unani medicine for its Qabiz(astringent) and Mujaffif (dessicant) properties in the treatment of bleeding disorders and diarrhea.[3] Samagh-e-Arabi, which is the gum of *Acacia nilotica* L. Wild ex. Del contributes to astringent property of the formulation and also acts as a binding agent for the tablet.[4]

The present study was aimed to standardize this important Unani drug in order to ensure its authenticity, quality and efficacy. The drug was prepared at laboratory scale at DSRU, New Delhi. According to the formulation composition of the drug, Qurs-e-Gulnar is composed of 6 ingredients (Table 1) in which 5 ingredients are of plant origin and one is mineral origin as described in NFUM-I.[1]

Table 1: Formulation composition.

S.No.	Ingredients	Botanical Name/English Name	Part used	Form
1.	Gulnar	*Punica granatum* L.	Flower	Powder
2.	Gil-e-Armani	Aluminium silicate	powder	Powder
3.	Samagh-e-Arabi	*Acacia nilotica* L. Wild ex. Del	Gum	Powder
4.	Gul-e-Surkh	*Rosa damascene* Mill	Flower	Powder
5.	Aqaqia	*Acacia nilotica* L. Wild ex. Del	Extract of pods	Powder
6.	Kateera	*Cochlospermum religosum* (L) Alston	Gum	Powder
7.	Aab-e-Gulnar	*Punica granatum* L.	Infusion	Liquid

In order to develop SOP and to evaluate the pharmacopoeial standards, the drug was subjected to microscopic, physicochemical and quality control analysis. The present paper describes the salient features of preparation, microscopic characters, physico-chemical

parameters, HPTLC, heavy metal estimation, aflatoxins, microbial load and pesticide estimation not reported so far to prove the scientific validation of the drug Qurs-e-Gulnar.

MATERIALS AND METHODS

Preparation of drugs

All the ingredients were procured from local raw drug dealer and were identified botanically[6,7] using pharmacognostical methods. The ingredients were further validated by comparing with the monographs available in UPI (Part I), Vol. III & IV.[8]

All the ingredients were taken of pharmacopoeial quality. The ingredients were cleaned and dried under shade to remove the moisture if any. The ingredients (S.No. 1 to 6, Table 1), were crushed separately in an iron mortar to obtain a coarse powder. The coarse powder was further ground in a mixer grinder to get the fine form. The fine powder was mixed thoroughly and sieved through mesh no. 80. Aab-e-Gulnar was added to the mixture and again mixed thoroughly to obtain the *lubdi* (mass). The Qurs were prepared from the *lubdi* (mass) and dried under shade. The prepared Qurs were stored in tightly closed glass container free from moisture and kept in a cool and dry place.

Microscopy

5gm of the powdered drug was taken and stirred gently with hot water in a beaker. The supernatant was discarded and the residue was washed with the distilled water. A little residue was stained with iodine solution and mounted in 50% glycerin. Some of the residue was heated in chloral hydrate solution and mounted in 50% glycerin and a little residue was boiled in 2% potassium hydroxide solution, washed with distilled water and mounted in 50% glycerin.[6,7,9]

Physico-chemical Analysis

The physico-chemical parameters of Qurs-e-Gulnar such as removal of foreign matters, moisture contents, extractive values (solubility in water, ethanol and hexane), ash values (total ash and acid insoluble ash), pH values (1% and 10% aqueous solution) and volatile oil estimation were analyzed by standard methods.[10, 11]

Quality control Analysis

Quality control parameters like microbial load, heavy metals, aflatoxins and pesticidal residues for the samples of the drug were undertaken and analyzed. The microbial load

estimation was carried out as per the guidelines.[12] Heavy metal analysis was done by atomic absorption spectrophotometer.[13] Analysis of aflatoxins was performed by TLC method.[12] Pesticide residues were analyzed using GC-MS Agilent instrument equipped with mass selective detector as per the methods of AOAC (2005)[13] and (Anonymous, 2000).[12, 14]

HPTLC Analysis

The prepared drug was extracted separately with chloroform and ethanol under refluxing conditions on a water bath for about 30 minutes and then filtered. The extracts were concentrated and made up to 10ml in a volumetric flask separately. These solutions were used for the HPTLC finger print analysis by employing CAMAG Linomat IV sample applicator on aluminum TLC plate pre-coated with silica gel 60 F_{245} (E. Merck). The plate was developed up to the distance of 8cm in the chamber (10x10), using 10ml of the developing system Toluene; ethylacetate (9:1) as mobile phase. The plate was dried at room temperature, observed and scanned under UV 254nm and UV 366nm. Further the plate was dipped in 1% vanillin-sulphuric acid reagent and heated at 105^{o} C till colored spots appeared.[5, 16, 17]

OBSERVATIONS

Qurs-e-Gulnar is a reddish brown tablet with mild pleasant odor and mucilaginous taste. The drug did not show any filth, fungus or objectionable matter while the sample was spread in a petri dish. (Fig.1).

Fig. 1: Qurs-e-Gulnar.

On examination under microspcope, following cells/tissues/cell contents were observed:

Gulnar, *Punica granatum* L (flower).

1. Abundant glandular trichomes consisting of uniseriate stalk and a globular head. Very occasionally, there is no stalk and the gland is sessile.
2. Fragmentof calyx in surface view consisting of thick walled parenchyma cells with glandular trichomes.
3. Fragment of epidermal cells of the petals in surface view consisting of slightly thick walled, parenchymal cells with slightly sinuous outline (Fig 2a,b,c).

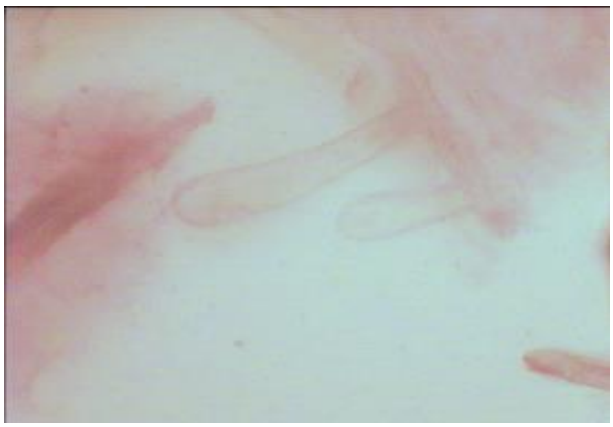


Fig. 2(a): X40 Trichomes of Gulnar.

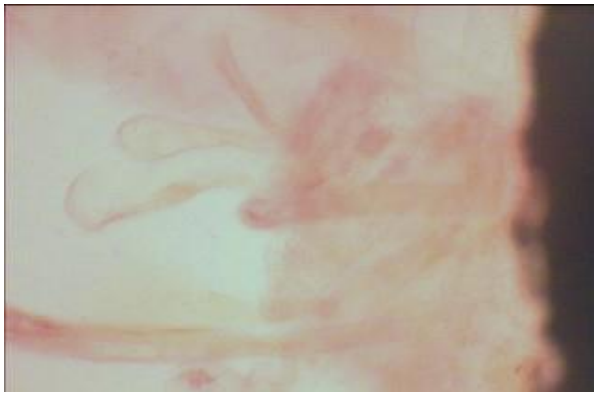


Fig. 2(b): X40 Fragment of sepal of Gulnar with Trichomes.

Fig. 2(c): X40 Fragment of Gulnar petal in surface view.

Gul-e-Surkh, *Rosa damascena* Mill (flower)

1. Fragment of epidermal cells of the petals in surface view appear rectangular or somewhat elongated, moderately thick walled with sinuous outline.
2. Fragment of sepals consisting of polygonal to oval parenchymal cells slightly thick walled having elongated trichomes.
3. Numerous long, unicellular, unseptate, non-glandular trichomes(Fig 2d,e,f).

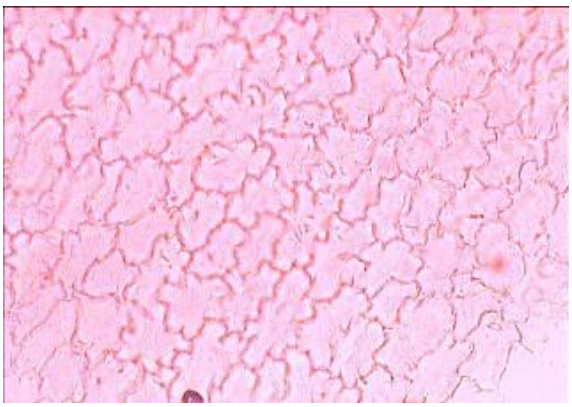


Fig. 2(d): X40 Epidermal cells of Gul-e-Surkh petal.

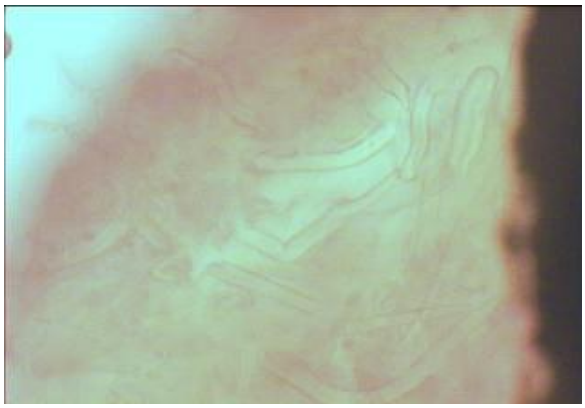


Fig. 2(e): X40 Fragment of Gul-e-Surkh sepal showing trichomes.

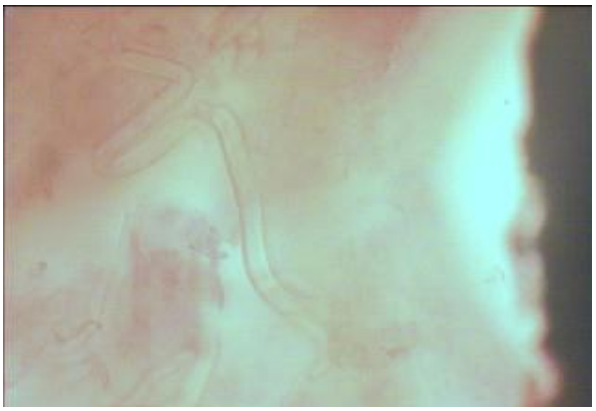


Fig. 2(f): X40 Elongated trichomes of Gul-e-Surkh.

The results observed for the physico-chemical data, microbial load, aflatoxins, pesticideresidues, heavy metals and HPTLC profile are shown in Table 2, 3,4,5,6 & 7 respectively.

Table 2: Physico-chemical Parameters.

S. No.	Parameters	Results (%)
1.	Water soluble extractive (%)	33.86-35.15
2.	Alcohol soluble extractive (%)	3.24-3.41
3.	Hexane soluble extractive (%)	0.23-0.28
4.	Loss in wt. on drying at 105^0C	8.86-9.57
5.	Total ash (%)	20.32-21.69
6.	Acid Insoluble ash (%)	12.17-13.57
7.	Water soluble Ash (%)	0.31-0.75
8.	pH of 1% aqueous Soln.	5.45-5.49
9.	pH of 10% aqueous Soln.	5.95-6.02
10.	Volatile oil	Traces

Table 3: Microbial load.

S. No.	Parameter Analyzed	Results	Permissible limit as per WHO
1.	Total Bacterial count	1 x10^2 cfu / gm	10^5 cfu / gm
2.	*Enterobacteriaceae*	Absent	Nil
3.	*Salmonella spp.*	Absent	Nil
4.	*Escherichia coli*	Absent	Nil
5.	*Staphylococcus aureus*	Absent	Nil
6.	*Pseudomonas aeruginosa*	Absent	Nil
7.	Total Fungal count	Less than 1 cfu/gm	10^3 cfu / gm

Table 4: Aflatoxins level.

S. No.	Parameter Analyzed	Results	Permissible limit as per WHO
1	B1	Not detected	< 2ppb
2	+B2+G1+G2	Not detected	< 5ppb

Table 5: Pesticide residue.

S. No.	Parameter Analyzed	Results	Permissible limit as per WHO (mg/kg)
1.	Alachor	BLQ	0.02
2.	Aldrin	BLQ	0.05
3.	Azinphos –methyl	BLQ	1.0
4.	Chlordane (cis & trans)	BLQ	0.05
5.	Chlorfenvinphos	BLQ	0.5
6.	Chlorpyrifos	0.030	0.2
7.	Chlorpyrifos-methyl	BLQ	0.1
8.	Cypermethrin	BLQ	1.0
9.	DDT	BLQ	1.0
10.	Deltamethrin	BLQ	0.5
11.	Diazinon	BLQ	0.5
12.	Dichlorvos	BLQ	1.0
13.	Dimethoate	BLQ	0.1
14.	Dieldrin	BLQ	0.03
15.	Endosulphan	BLQ	3.0
16.	Endrin	BLQ	0.05
17.	Ethion	BLQ	2.0
18.	Fenitrothion	BLQ	0.5
19.	Fenvalerate	BLQ	1.5
20.	Heptachlor	BLQ	0.05
21.	Hexacholobenzene	BLQ	0.06
22.	Lindane (gamma HCH)	BLQ	0.6
23.	Malathion	BLQ	1.0
24.	Parathion	BLQ	0.5
25.	Parathion-methyl	BLQ	0.2
26.	Permethrin	BLQ	1.0
27.	Phosalone	BLQ	0.1
28.	Primiphos methyl	BLQ	0.1

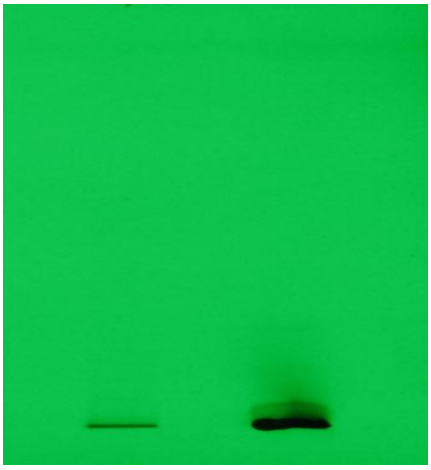
BLQ-Below Limit of Quantification

Table 6: Heavy Metals.

S. No.	Parameter Analyzed	Results	Permissible limit as per WHO (ppm)
1.	Lead	Not detected	10.00
2.	cadmium	Not detected	0.30
3.	Arsenic	Not detected	3.00
4.	Mercury	Not detected	01.00

Table 7: HPTLC Results.

S. No.	Extract	Solvent system	Developing reagent	R_f values with color		
				UV 254nm	UV 366nm	After derivatization
1.	Chloroform	toluene: ethyl acetate (9:1)	1%vanillin-sulphuric acid	No band	No band	0.05 (black)
						0.09 (purple)
						0.13 (light purple)
						0.23 (purple)
						0.29 (very light purple)
						0.36 (very light purple)
						0.88 (very light purple)
						0.95 (light purple)
2.	Ethanol	toluene: ethyl acetate (9:1)	1% vanillin-sulphuric acid	0.05 (green)	0.06 (blue)	0.0 (black)
					0.26 (blue)	0.08 (brown)
					0.35 (blue)	0.11 (purple)
						0.15 (light purple)
						0.24 (light purple)
						0.31 (very light purple)
						0.38 (very light purple)
						0.89 (light pink)
						0.95 (dark pink)



Chloroform ext. Ethanol ext.

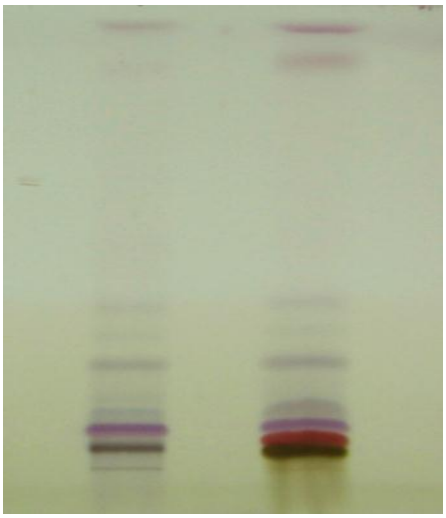
UV 254nm

Fig. 3(a)

Chloroform ext. Ethanol ext.

UV 366nm

Fig. 3(b)



Chloroform ext. Ethanol ext.

After Derivatization

Fig. 3(c)

RESULTS AND DISCUSSION

Physico-chemical Analysis

The physico-chemical data of the drug Qurs-e-Gulnar are shown in Table 2. The extractive values show that the solubility of phytoconstituents of the drug was more in water (33.86-35.15%) and less amount of phytoconstituents are soluble in alcohol (3.24-3.41%) and Hexane (0.23-0.28%). The moisture content in drug was low as the loss in weight on drying at 105^{O} C occurred below 10%. A high value of total ash (20.32-21.69%) and Acid insoluble ash (12.17-13.57%) indicates that the drug contains siliceous matter. The water soluble ash

value is low (0.31-0.75%). The aqueous extract of the drug was slightly acidic as pH of aqueous solution falls in the range of 5-6. The volatile oil is in traces only.

Quality Control Analysis

Microbial load

Microbial content of the drug is given in Table 3. The estimation gives the tentative idea to assess the quality and safety of the drug prepared. The assessment is done for estimating the total viable count of bacteria, total fungus count, count of bacteria belonging to the *Enterobacteriaceae* family, count of pathogens like *E. coli*, *Staphylococcus aureus* and *Salmonella spp.* The results indicate the microbial load to be within the permissible limit prescribed by WHO indicating that the drug is safe for internal use for the treatment of prescribed aliments.

Aflatoxins

The results of aflatoxins in the drug are given in Table 4. Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus, A. parasiticus* and *A. nomius*. The results do not show any evidence for the presence of any of the aflatoxin contents (B1, B2, G1, and G2).

Pesticidal residues

The results of pesticidal residues are given in table 5. Production of herbal drugs according to good agricultural practices with no pesticides is very difficult due to several factors. Estimation of pesticides in the samples became a major task and the drug was analyzed using GC-MS (detection limit up to 0.01ppm). The results indicated the drug to be free of pesticide residues and safe for use.

Heavy metal Analysis

The results of Heavy metal estimation are given in Table 6. Heavy metals are hazardous to human and animal health, their content in any drug used for consumption or medicinal purpose must be limited. The heavy metal content in Qurs-e-Gulnar was found to be within the permissible limit of WHO & PCIM&H, indicating that the drug is safe and free from any type of heavy metal contamination.

HPTLC Profile

The results of HPTLC Profile are given in Table 7. HPTLC Profiling is very reliable and convenient for identification of crude drugs as well as compound formulations as plant species produces a distinct chromatogram. HPTLC photograph of Qurs-e-Gulnar with both solvent system was observed under UV 254nm, UV 366nm and after derivatization. The chromatogram of chloroform extract shows no band under UV 254nm and UV 366nm and shows 8spots after derivatization. The chromatogram of ethanol extract shows 1 spotunder UV 254nm, 3 spots under UV 366nm and 9 spots after derivatization. (Fig.3a,3b&3c).

CONCLUSION

It can be concluded that organoleptic parameters are not much reliable in identification of herbal drug as the ingredients are powdered and mixed together for preparing compound formulation. The present study therefore holds high significance as the microscopic features; various physico-chemical parameters, HPTLC profile etc. provide criteria for easy identification of the drug Qurs- e-Gulnar and quality control analysis ensure the authenticity, quality and efficacy of the medicine.

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