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Standardization and HPTLC Fingerprinting of a Polyherbal Unani Formulation

Mirza Belal Beg[1*], Uzma Viquar[2], Mohammad Abdul Rasheed Naikodi[3], Habiba Suhail[4], Munawwar Husain Kazmi[5]

[1]MD Scholar, P.G. Department of Ilmul Advia (Pharmacology), National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India, [2]Associate Professor, Department of Ilmul Advia (Pharmacology), National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India, [3]Research Assistant, Drug Standardization Research Unit, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India, [4]MS Scholar, Department of Ilmul Qabalat wa Amraze Niswan (Obstetrics and Gynaecology), National Institute of Unani Medicine, Bengaluru, India, [5]Professor & HoD, Department of Ilmul Advia (Pharmacology), National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.

ABSTRACT

Background: The Unani system of medicine has been practised since centuries for the treatment of a range of diseases. In spite of their efficacy they have been widely criticised due to the lack of standardization and poor quality control. Standardization of Unani medicine is a valuable issue at the present because they are very prone to contamination, deterioration, adulteration and variation in composition due to biodiversity as well as careless collection.

Objective: To Standardize and Development of HPTLC Fingerprinting of a polyherbal Unani formulation *Qurs-e-Safa*.

Materials and methods: The conventional and modern analytical techniques were used to standardise *Qurs-e-Safa*. The study was carried into three different batches of *Qurs-e-Safa* prepared with its ingredients. The parameters studied are organoleptic, microscopic, physicochemical parameters, phytochemical screening, TLC, HPTLC profile, aflatoxin, microbial load and heavy metal analysis.

Results and conclusion: *Qurṣ-e-Saʹfa* is dark yellow in colour and aromatic smell. Uniformity of diameter and weight variation were found to be 13 ± 0, and 524.7 ± 1.72 mg. friability, hardness and disintegration time of all 3 batches were found to be (0.0615 ± 0.004, 0.0885 ± 0.0047 and 0.0725 ± 0.0058), (3.5 ± 0.2886, 3.67 ± 0.1674 and 3.67 ± 0.1674) and (16 to 17 minutes). Extractive value were found to be maximum in distilled water (38.488 ± 0.20, 37.3824 ± 0.38 and 39.8177 ± 0.13) followed by alcohol (27.5406 ± 0.54, 27.5656 ± 0.32 and 26.9229 ± 0.25). Loss of weight on drying, pH, total ash, acid insoluble ash, qualitative test was set in. Phytochemical screening revealed the presence of Carbohydrates, Phenols, Resins, Proteins, Steroids, fixed oil and Flavonoids. The microbial load was found absent and heavy metals were within permissible limits. The data evolved from the study may serve as a reference to validate and also help in the quality control of other finished products in future research.

Keywords Standardization, Physicochemical analysis, *Qurs-e-Safa*, HPTLC, Unani Medicine.

INTRODUCTION

Herbal drugs are used since ancient times as medicines for the prevention and treatment of various diseases. Herbal drug make an important contribution to health care in spite of significant development seen in modern medicine in recent decades (Calixto, 2000). In India where various traditional system of medicine are existed simultaneously. Unani system of medicine is one of them which are based on plant, animal and mineral origin drug which are used either as a single or in compound formulation (Kadam et al., 2012). Which was evolved through day to day life experiences upto proper research and progress successfully along with other system of medicine for the betterment of humankind (Mukherjee and Wahile, 2006).

Nowadays, herbal medicine expands rapidly all over the world due to the consciousness of health problem associated with the synthetic medicine. Mostly medicinal plants are highly recognised as a wide source of therapeutic agent for the prevention and treatment of the disease (Satakopan, 1994). In India the popularity of herbal drugs are increasing day by day in rural as well as urban community because of its efficacy, safety and easily availability due to this there is increase in their demand leading to decline in their quality because of adulteration, substitution and lack of knowledge about cultivation, collection and storage etc. (Gadre et al, 2001). WHO recommended significant guidelines for standardization of herbal formulation and the aim of WHO guidelines is to determine basic criteria for the assessment of quality, efficacy

[*]Correspondence: Mirza Belal Beg
E-mail: belalmirza7223@gmail.com
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and safety of herbal medicine (Anonymous, 1991; Phillipsion, 1989).

Qurs is a dosage form used in Unani system of medicine described by Unani physician *Indarumakhas* (Rahman, 2010). In present study *Qurs-e-Safa*(QS) a Unani compound formulation mentioned in various Unani classical books including *Al-Qarabadeen* (Kabeeruddin, 2006); *Qarabadeen-iQadri* (Anonymous, 2005), *Qarabadeen-iAzamwaAkmal* (Arzani, 2009); *Kamil-us-Sanat* (Anonymous, 2010). QS are use externally in many skin diseases like Safa (*cicatricial alopecia*), *Quba* (dermatophytic infections) including various types of fungal infections such as *Quba-iRas* (*tineacapitis*), *Quba-iZukhn* (*tineabarbae)*, *Quba-iAzfar* (*tineaungium*), *Quba-iBadan* (*tinea corporis*), *Quba-iAejan* (*tineacruris*), *Quba-iYad* (*tinea manus)*, *Quba-iQadam* (*tineapedis*), *SudaBalghami* (headache due to phlegm) and *SardAwram* (inflammation caused by phlegm and black bile humors) (Kabeeruddin, 2006; Anonymous, 2005; Arzani, 2009; Anonymous, 2010; Baghdadi, 2004; Rushd, 1987).

QS composed of *Zard-i-chob* (*Curcuma longa* L.), *Muqil* (*Commiphora mukul*) and *Badam Talkh* (*Prunus amygdalus* var. *amara*) and is prepared by mixing all the three ingredients in *sirka* (Vinegar) to make *Qurs* (tablet). *Qurs* smashed and mixed with *Sirka-i Anguri* (grape vinegar) or *Aab-e-Kasni* and apply externally (Kabeeruddin, 2006; Anonymous, 2005; Arzani, 2009; Anonymous, 2010).

The formulation had not been standardized so far on organoleptic, microscopic, physicochemical parameters, phytochemical screening, TLC, HPTLC profile, aflatoxin, microbial load and heavy metal analysis. The present study was designed to develop the various physico-chemical standards of QS to assess the genuine quality of the formulation as it was used by traditional practitioners for past many decades which can be used as a quality control tool.

MATERIALS AND METHODS

Collection of material: Plant Ingredients of the formulation QS were procured from the GMP certified pharmacy, identified and authenticated by the botanist at National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.

Preparation of formulation: QS was prepared as per the prescribed procedures mentioned in the *Al Qarabadeen* (Kabeeruddin, 2006); Unani Pharmacopoeia of India (Anonymous, 2010).

Ingredient	Botanical name	Part used	Quantity
Zard chob	*Curcuma longa* L.	Dried rhizome	One part
BadamTalkh	*Prunus amygdalus* var. amara	Kernel	One part
Muqil	*Commiphora mukul* (Hook ex Stocks) Engl.	Exudate	Two part
Sirka	Vinegar	Liquid form	Quality sufficient

According to pharmacopoeia procedures and standardization, QS has to be study for physicochemical evaluation along with organoleptic properties like appearance, colour, odour, taste and texture, diameter, weight variation, hardness of tablet, disintegration time, ash values, water and alcohol soluble matter, determination of pH (1% and 10% solution), loss in weight on drying at 105°C, bulk density. The powder microscopy of QS was carried out and examined for lignified structures like stone cells, calcium oxalate crystal, starch granules, epidermal cells, xylem fibres, tracheids, parenchyma cells, cuticular cell walls, essential oils, resins, fats and fatty oils. Friability of tablets was determined by the help of Roche's Friability test apparatus. Three different batches of powdered samples of QS were evaluated for total ash, acid insoluble ash, water and alcohol soluble matter.

The microbial load analysis was carried out in three different samples of QS. The media used are Soyabean Casein Digest Agar Media, Sabouraud Dextrose Agar with Chloramphenicol Media, HiCrome™ E. coli Agar Media, Hi CromeRajHans Medium, Modified (Salmonella Agar, Modified). The aflatoxin determination for aflatoxins B1, B2, G1 and G2 are studied. Heavy metal analysis was carried out at Drug Standardization Research Institute (DSRI), Ghaziabad, India for the detection of lead (Pb) cadmium (Cd), arsenic (As) and mercury (Hg).

High Performance Thin Layer Chromatography (HPTLC) analysis

Alcoholic extract of QS was studied for thin layer chromatography and HPTLC and evaluated under various detection system viz., UV 254nm, UV 366nm and upon derivatizion with anisaldehyde sulphuric acid at 580nm. Five gram of powdered sample was taken and reflux with 200 ml of alcohol using soxhlet apparatus on a water bath for 30 min. The content of the flask was filtered, and the obtained extract was concentrated to 5 ml and used as a sample for thin layer chromatography. Thin layer chromatography was carried out on pre-coated Silica gel aluminium plates. The sample alcoholic extract was applied on the TLC plate and developed with the selected mobile phase. The R_f values for the spots obtained in the TLC plate are calculated.

Method conditions

Made/ Make of Instrument	: DesagaSarstedtGruppe (Germany),
Development Chamber	: 20 X10 cm, Twin-trough chamber
Stationary phase	: Pre coated silica gel 60 F_{254}:aluminium plates (Merck, KgaA, Germany)
Plate thickness	: 0.2 mm
Plate size	: 200 x 100 mm
Distance from starting	: 20 mm
Distance from bottom	: 10 mm
Volume applied	: 5 µl
Band length	: 10 mm
Distance between tracks	: 20 mm
Development distance	: 80 mm
Solvent used	: HPLC grade
Extract storage vials	: 5 ml glass vials
Mobile phase Used	: Toluene: Ethyl Acetate: Methanol (7:2:1, v/v/v)

RESULTS AND DISCUSSION

Organoleptic properties are of very important parameters for quick identification of the drug and helps to determine the quality of drugs. The organoleptic characteristics of QS such as appearance as slightly biconvex tablet-like, dark yellow colour due to presence of curcumin as major chemical constituents in *zard chob*, aromatic odour due to essential oil presence in *zard chob* and *muqil*, hard in texture. The powder microscopy of QS revealed the presence of epidermal cells, xylem fibres, tracheids, stone cells (*Badam Talkh*) and parenchyma cells filled with amorphous masses of gelatinized starch grains (*Zard chob*) as shown in (Fig. 5).

Uniformity of diameter of QS was found to be 13mm. Weight variation test is done to ensure that QS contain the equal amount of drug in each tablet. No tablet was found out of the limit of 5%. Hence, the percentage weight variation of QS was found to be within the prescribed pharmacopoeial limits of 5%. The average weight of QS was found to be 524.7 mg ± 1.72. Friability test was done to find out the possible reduction in the weight of QS as a result of mechanical erosion during handling. The mean percentage of friability of QS was found to be 0.07 ± 0.01%, which is very good in comparison to the maximum permissible limit of 0.5 to 1%. Hardness test was done to measure the resistance of QS to mechanical deforming. The mean value of hardness of QS was found to be 3.61 ± 0.33 kg/cm, which is near about 4 kg/cm, the minimum acceptable hardness for uncoated tablets. Disintegration test was carried out to determine whether QS disintegrates within the prescribed time when placed in a liquid medium. Majority of the herbal tablets have maximum disintegration time of around 30 min. The mean value of disintegration time in aqueous medium of QS was found as 17 ± 0.71 minutes which is near about to 30 min (Lachman, Lieberman and Kanig, 1987).

The ash value of the drug is the residue remaining after incineration it usually determines the inorganic substances present in the drug. Similarly, it can also detect the nature of the material, which was added to the drug for the purpose of adulteration. Hence, determination of ash value provides a basis for determining the identity and purity of the drug. The mean percentage values and standard deviation of the total ash and acid insoluble ash was found to be 10.01 ± 0.18% and 5.07 ± 0.26 % respectively (Anonymous, 2010).

Extractive value of a drug in a definite solvent is an index of purity of a drug and plays a major role to determine adulteration (Anonymous, 1998).The mean percentage values and standard deviation of alcohol and water soluble matter of QS was found to be 27.34 ± 0.66% and 38.56 ± 1.13% respectively.

The mean percentage of bulk density of QS was found to be in the range of 0.80 to 0.81 in three different batches. The pH value of drug is also a significant parameter. pH value of QS in aqueous solution was found to be as 6.03 in 1% aqueous solution and 6.11 in 10% aqueous solution and both were weakly acidic (Anonymous, 2006).

Loss of weight on drying at 105°C was carried out to determine the amount of water or volatile matter present in QS, which was removed during drying, the mean percentage of loss of weight on drying at 105°C of QS was found to be 6.75 ± 0.04%. As the QS contain less moisture upto 7% and therefore having very less chance of microbial growth (Anonymous, 2010). The corresponding physico-chemical parameters analysed, results are shown (Table 1).

The phytochemical screening of drug useful in finding the chemical nature of the drug that will reveal the presence of metabolites such as alkaloids, glycosides, flavonoids and saponins etc. In the present study, a systematic phytochemical screening of aqueous and alcoholic extract of QS was carried out for the qualitative determination of different chemical constituents present in the drug extract. Different chemical tests are performed to detect the presence of nature of Phyto-constituents. The qualitative tests revealed the presence of carbohydrates, phenols, resins, proteins, steroids, flavonoids and fixed oils in the sample whereas alkaloids, glycosides, saponins, starch and tannins were found to be absent in the QS extracts and the findings are shown (Table 2).

The results of total bacterial load and total fungal count of the microbial studies are within the permissible limits, and other parameters was found to be absent in the drug. Quantitative determinations of heavy metals in herbal drugs are very important. If high quantity of heavy metals found present, this may lead to a number of health hazards. Hence, it was recommended by WHO that every herbal products or mineral-based drugs should be examined for the heavy metal contamination for toxic effects. In the formulation, heavy metals (lead, mercury, cadmium, arsenic) were found absent and complies with the permissible limit prescribed by WHO guidelines, indicating that the formulation is free from any unwanted contaminations and safe for consumption. Studies of aflatoxins, heavy metal analysis showed that the drug was free from contaminations. The present findings for microbial load, aflatoxin contamination and heavy metal analysis are shown in (Table 3-5).

Table 1. Physico-chemical parameters of the compound formulation QS

Parameters	Batch-I	Batch-II	Batch-III	Mean ± SD
Uniformity of diameter(mm)	13.00	13.00	13.00	13.0 ± 0.00
Friability (%)	0.05 - 0.07	0.07 - 0.08	0.07 - 0.08	0.07 ± 0.01
Hardness (kg/cm)	3 - 4	3.5 - 4	3.5 - 4	3.61 ± 0.33
Disintegration time in aq. media (min)	16 - 17	17 - 18	17 - 18	17 ± 0.71
Bulk density	0.80 - 0.81	0.81- 0.82	0.81 - 0.82	0.81 ± 0.01
Total ash (%w/w)	9.79 - 10.08	9.84 - 10.21	9.78 - 10.25	10.01 ± 0.18
Acid insoluble ash (%w/w)	4.58 - 5.34	4.99 - 5.35	4.88 - 5.33	5.07 ± 0.26
Alcohol sol. matter (%w/w)	26.98 - 28.62	27.05 - 27.49	26.53 - 27.38	27.34 ± 0.66
Water sol. matter (% w/w)	38.27 - 38.88	36.65 - 37.92	39.55 - 39.96	38.56 ± 1.13
pH of 1% aq. solution	6.01 - 6.02	5.99 - 6.01	6.04 - 6.09	6.03 ± 0.03
pH of 10% aq. solution	6.04 - 6.12	6.16 - 6.21	6.06 - 6.09	6.11 ± 0.06
Loss of weight on drying at 105^0C (%w/w)	6.75 - 6.79	6.67 - 6.76	6.70 - 6.78	6.75 ± 0.04

Table 2. Analysis of phytochemical constituents of the QS

Phytochemical constituents	Observations	
	Ethanolic extract	Aqueous extract
Alkaloid	_	_
Carbohydrates	+	++
Flavonoids	++	-
Fixed oil	+++	+
Glycosides	_	_
Phenols	+	_
Protein	-	+
Resins	++	+
Saponin	-	_
Starch	-	_
Steroids	+	-
Tannins	-	_

Table 3. Microbial Contamination

S.N	Parameter analysed	Results			Permissible limits as per WHO
		Sample-1	Sample-2	Sample-3	
1	Total Bacteria Load	07 X 10^2	08 X 10^2	05 X 10^2	Not more than 10^5/g
2	Salmonella spp.	Nil	Nil	Nil	Nil
3	Escherichia coli	Nil	Nil	Nil	Nil
4	Total Fungal count	Nil	Nil	Nil	Not more than 10^3/g

Table 4. Aflatoxin Contamination

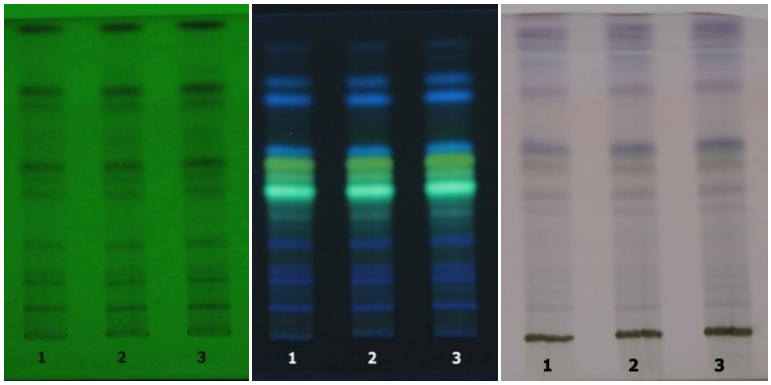
S.N	Parameter analysed	Results			Permissible limits as per WHO
		Sample-1	Sample-2	Sample-3	
1	B1	Nil	Nil	Nil	Not more than 0.50 ppm
2	B2	Nil	Nil	Nil	Not more than 0.10 ppm
3	G1	Nil	Nil	Nil	Not more than 0.50 ppm
4	G2	Nil	Nil	Nil	Not more than 0.10 ppm

Table 5. Heavy Metals detection

S.No	Parameters analysed	Results	UPI/WHO Permissible Limits
1	Lead- (Pb)	ND	Not more than 10 ppm
2	Cadmium- (Cd)	ND	Not more than 0.3 ppm
3	Arsenic- (As)	ND	Not more than 3.0 ppm
4	Mercury- (Hg)	ND	Not more than 1.0 ppm

HPTLC analysis of an alcoholic extract of study formulation

HPTLC analysis of QS was carried out in alcoholic extract and the sample applied on silica gel G pre-coated aluminium TLC plate and developed with selected solvent system of toluene: ethyl acetate: methanol (7:2:1, v/v/v) as a mobile phase and TLC plate was developed and detected using the UV visible chamber which clearly showed fourteen spots at UV 366nm in the densitogram as depicted (fig.1). The corresponding R_f values of the fourteen components are 0.07 (blue), 0.11 (blue), 0.15 (blue), 0.18 (blue) 0.20 (blue), 0.28 (blue), 0.35 (light green), 0.42 (green), 0.50 (green), 0.54 (yellow), 0.57 (blue), 0.74 (blue), 0.78 (blue) and 0.90 (blue); and under UV 254 nm shows nine spots at R_f values 0.05, 0.11, 0.25, 0.42, 0.48, 0.52, 0.72, 0.78 and 0.97 (all black); and under visible region after derivatizing with anisaldehyde sulphuric acid and heating at 105^0C shows eight spots at R_f values 0.07, 0.38, 0.42, 0.50, 0.57, 0.74, 0.85 and 0.92 (all purple). The TLC plate upon scanning under densitometer to obtained the corresponding HPTLC densitograms having peak areas for the spots. The corresponding data for the densitograms obtained as shown in (Fig. 2-4) are presented in (Table 6-8).



At UV 254nm | At UV 366nm | After derivatization with anisaldehyde acid

Fig.1 TLC of alcoholic extract of QS

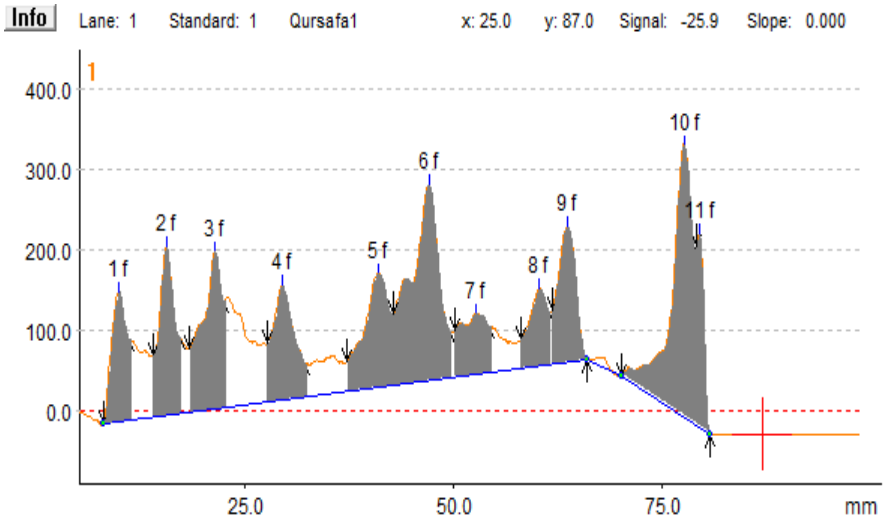


Fig.2 Densitogram of alcoholic extract of QS at UV 254nm

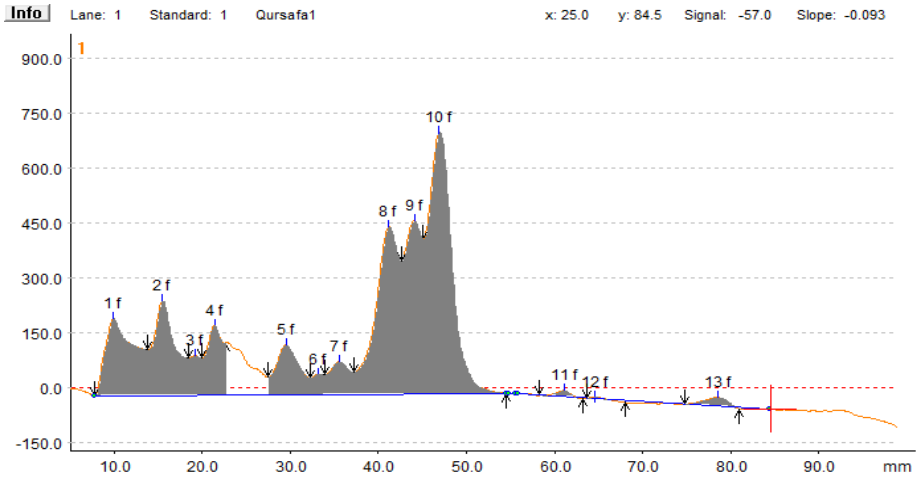


Fig.3 Densitogram of alcoholic extract of QS at UV 366nm

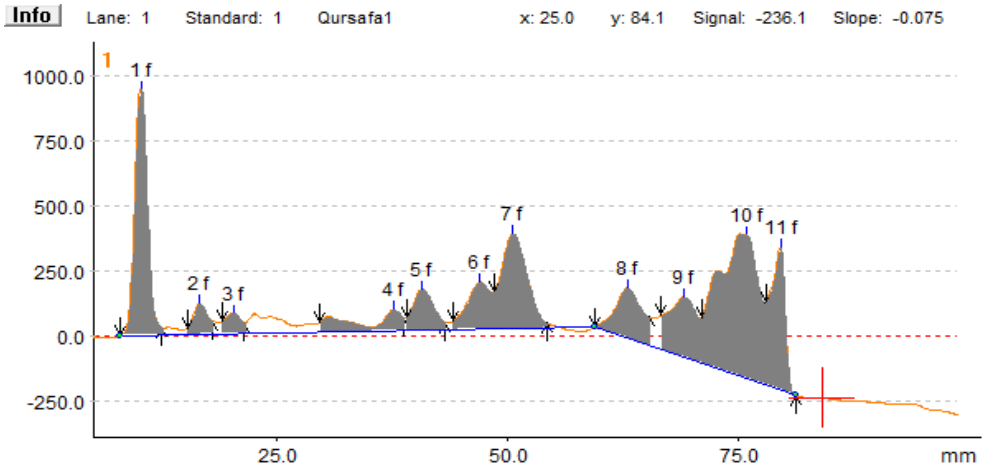
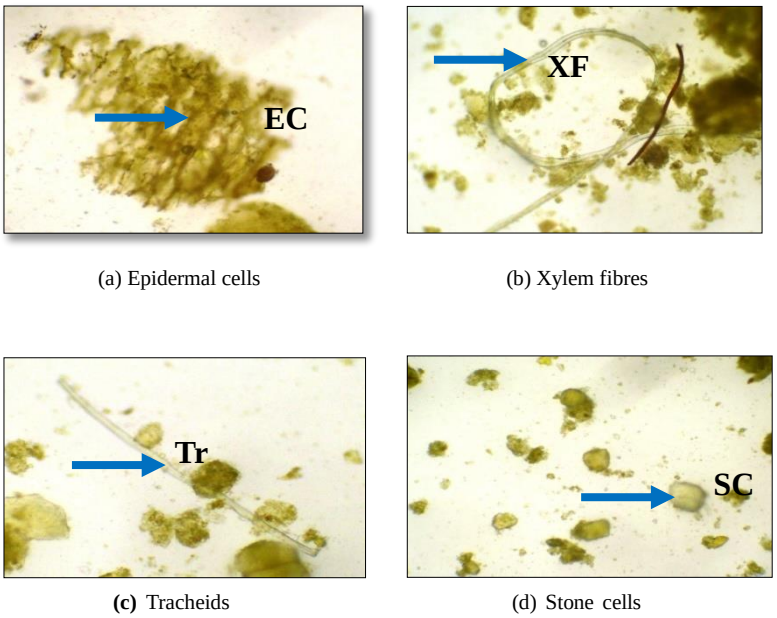


Fig.4 Densitogram of alcoholic extract of QS after derivatizion with anisaldehyde sulphuric acid.



(a) Epidermal cells (b) Xylem fibres

(c) Tracheids (d) Stone cells

Fig.5 Powder Microscopy of QS

Table 6. Peak list of alcoholic extract of QS at UV 366nm

Peak no	Y-Pos	Area	Area%	Height	R_f value
1	9.9	787.65	10.53	208.72	0.01
2	15.5	718.92	9.62	254.92	0.09
3	19.2	155.27	2.08	106.76	0.14
4	21.4	389.17	5.20	187.41	0.17
5	29.5	410.81	5.49	134.23	0.28
6	33.2	78.97	1.06	55.83	0.34
7	35.5	233.05	3.12	88.85	0.37
8	41.2	1370.11	18.32	450.35	0.45
9	44.1	1061.42	14.20	465.40	0.49
10	46.9	2166.69	28.98	703.22	0.53
11	61.1	28.90	0.39	15.88	0.72
12	64.6	5.32	0.07	4.08	0.77
13	78.5	70.65	0.94	23.12	0.97

Table 7. Peak list of alcoholic extract of QS at UV 254nm

Peak no	Y-Pos	Area	Area %	Height	R_f value
1	9.8	330.46	6.18	161.89	0.01
2	15.5	442.67	8.28	209.82	0.09
3	21.3	540.81	10.11	196.55	0.17
4	29.4	431.13	8.06	142.54	0.28
5	40.9	492.06	9.20	142.23	0.44
6	47.0	974.64	18.23	243.19	0.53
7	52.6	282.63	5.29	76.31	0.61
8	60.3	245.73	4.60	96.08	0.71
9	63.6	411.22	7.69	168.13	0.76
10	77.7	969.55	18.13	336.22	0.95
11	79.5	226.09	4.23	237.87	0.98

Table 8. Peak list of alcoholic extract of QS after derivatized with anisaldehyde sulphuric acid at 580nm.

Peak no	Y-Pos	Area	Area %	Height	R_f value
1	10.2	1491.49	15.74	941.54	0.02
2	16.6	209.36	2.21	118.48	0.11
3	20.3	154.39	1.63	79.69	0.16
4	37.6	394.10	4.16	82.36	0.40
5	40.7	412.36	4.35	158.36	0.44
6	47.0	504.92	5.33	182.24	0.53
7	50.5	1169.14	12.34	363.44	0.58
8	63.1	646.31	6.82	193.93	0.75
9	69.2	778.13	8.21	231.66	0.84
10	75.9	2750.83	29.04	549.79	0.93
11	79.6	961.75	10.15	546.37	0.98

CONCLUSION

It can be concluded that the so-called traditional herbal wealth can be converted into gold as far as India is concerned. This is possible if we establish proven efficacy of these medicines on scientific lines; failing this, the system may be able to survive for long only as the role of its golden past. Standardization of QS present unique evidence-based research in the study as no data was previously available. The formulation QS was standardized for the first time to generate various standard parameters of QS. The results obtained for the standardization parameters may serve as a reference standard and helpful in production of efficacious Unani formulation in future.

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CONFLICT OF INTEREST

There are no conflicts of interests.

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