Standardization of Qurs-e-Ghafis: A Polyherbal Unani Formulation

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Abstract

Qurs-e-Ghafis (QG) is a compound formulation (tablet) of Unani medicine, mainly used in hepato-biliary disorders. It contains three drugs of plant origin that are described in Unani literature to possess hepatoprotective effect. In experimental studies QG and its ingredients have shown to possess hepatoprotective effect. Studies for the identity and quality assurance of the ingredients have also been conducted but the compound drug as such has not been standardized on physico- chemical and phytochemical parameters so as to ensure its quality. Therefore, in the present study QG was studied on certain physic-chemical and phytochemical parameters to determine the standards of its quality and purity. The parameters and protocol recommended for testing the AYUSH drugs were followed in this study.

Key Words: *Qurs-e-Ghafis,* Standardization, Phytochemical, Unani drugs, Quality control.

Introduction

Qurs-e-Ghafis (QG) is a pharmacopoeial preparation described to be hepatoprotective and useful in a number of liver diseases (Khan, 1921). It contains extract of *Gul-e-Ghafis (Agrimonia eupatoria),* Dried Rhizome of *Sumul-ut-teeb (Nardostachys jatamansi) and Tabasheer* (a silicasious matter collected from *Bambusa arundinasea)* in the ratio of 5:2.5:1 and prepared in tablet form. In an experimental study QG has been shown possess significant hepatoprotective effect (Anas, 2010). Its ingredients though have been studied on physic chemical parameters but, the compound drug has not been studied so far on the parameters of standardization therefore no physico-chemical standards are available to assess its purity and quality. The data of individual ingredients cannot be extrapolated as a matter of principle, to set the standards of compound formulation. Therefore the standardization of compound drug (the dosage form of tablet) is necessary. In view of the above therefore present study has been designed to fix the various physico-chemical standards to assess the quality the formulation.

The physico-chemical attributes selected for the purpose of standardization included the determination of (i) Weight; (ii) Diameter and Thickness of tablet; (iii) Disintegration Time; (iv) Friability; (v) Successive Extractive Values; (vi) Water and Alcohol Soluble Contents by Cold and Hot Method; (vii), Moisture Contents; (viii) Ash values; (ix) Loss of weight on drying; (x) pH of 1%

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and 10 % solution; (xi) Thin layer chromatography (TLC) and; (xii) Qualitative analysis for various chemical constituents. The methodology was devised following the guidelines suggested for herbal and AYUSH drugs (Anonymous, 1968; 1970; 1989).

Materials and Methods

Ingredients of *Qurs-e-Ghafis*

As described in *Ilajul-Amraz* (Khan, 1921) QG contains following ingredients:

Agrimonia eupatoria	(Extract of *Gul-e-Ghafis*)	70 g
Nardostachys jatamansi	(Dried Rhizome *of Sumul-ut-teeb*)	35 g
Bambusa arundinasea	(*Tabasheer*, a silicasious matter)	14 g

Collection of raw materials

The crude drugs were procured from local market (Bara Dwari) of Aligarh. Pharmacognosy section of Department of Ilmul Advia, Aligarh Muslim University, Aligarh confirmed the identity and purity of the drug samples. Water extract of *Gul-e-Ghafis* was prepared with the help of Soxhlet apparatus.

Preparation of the Qurs

All the ingredients, except the extract of *Gul-e-Ghafis,* were powdered in an electric grinder. The powders of the two ingredients were mixed together along with extract of *Gul-e-Ghafis* and granules were prepared from it. The dried granules were passed through the compressing machine to get the tablets of 500 mg (Anonymous, 1968; 1970). The tablets were prepared at Dawakhana Tibbiya College, AMU, Aligarh with the help of a semi automatic tablet making machine.

Physicochemical Parameters

Weight and diameter variation test of tablet was conducted by the method of Dandagi *et al* (2006), while determination of disintegration time and friability test were carried out by the method mentioned in “Food and Drug Regulations” Ministry of Health, U.S.A (Anonymous, 1989) and by Vijay and Mishra (2006), respectively. Extractive values, water and alcohol soluble contents, loss of weight on drying, ash values (total ash, water soluble ash, acid insoluble ash) and pH of 1% and 10% aqueous solution were determined by the methods prescribed in British Pharmacopoeia and Physico-chemical Standards of Unani

Formulations, respectively (Anonymous, 1968; 1987). Moisture content was determined by the method of Jenkins *et al*., 1967.

Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out on T.L.C. aluminium plates pre-coated with silica gel 6 of 254 (layer thickness 0.25mm) for two extracts of QG viz. Pet. ether and chloroform. Chromatography was conducted using different organic solvent systems. The solvent systems used for petroleum ether extract and chloroform extract were Petroleum ether + Diethyl ether (3:2) and Chloroform + Benzene (4:1), respectively. The plates were later sprayed by different spraying reagents. The *Rf* values of the spots were calculated by the following formula:

R_f value = Distance travelled by the spot / Distance travelled by the solvent (Anonymous, 1968).

Qualitative Analysis of Chemical Constituents

The qualitative analysis of different chemical constituents likely to be present in *Qurs-e-Ghafis* was carried out according to the scheme proposed by Bhattacharjee and Das (1969). Various tests for the qualitative estimation of alkaloids, glycosoids, amino acid, flavonoids, phenols, proteins, resins, sterols/ terpenes, sugars, tannins were carried out by standard methods.

Observations and Result

The data is based on multiple observations.

The colour of QG was found to be blackish brown. Its shape was round flat and appeared like a tablet. It had a hard texture, bitter taste and agreeable smell (Table 1).The mean value of weight of QG was measured to be 500.1 ± 3.37 mg (Table 2). The mean values of the diameter and thickness were 13.50 ± 0.03 mm and 4.50 ± 0.03 mm, respectively (Table 3). The mean values of disintegration time in water and in a medium simulating with gastric fluid were found to be 16 ±0.57 seconds and 12.33 ±0.88 seconds, respectively (Table 4). The mean percentage of friability was 1.80 ± 0.02 (Table 5). The mean percentage of alcohol and water soluble contents was 34.49 ± 0.28 and 22.94 ± 3.79, respectively (Table 6)**.** The mean of the pH value of 1% and 10% solution was found to be 5.38 ± 0.36 and 5.37± 0.01, respectively (Table 7). The mean percentage of the moisture content was 7.53± .14 (Table 8). The mean percentage of total ash, acid insoluble ash and water soluble ash was

12.48 ± 0.27, 8.01 ± 0.06 and 2.14 ± 0.09 (Table 9). The mean percentage of loss of weight on drying was 7.43 ± 0.69 (Table 10). The mean percentage of successive extractive values were recorded as 2.46 ± 0.082.59 ± 0.08, 1.43 ± 0.06, 0.74 ± 0.06, 2.21 ± 0.04 and 8.06±0.39 with petroleum ether, diethyl ether, chloroform, benzene, alcohol and water, respectively (Table 11).

The R_f values calculated after visualizing the spots of each plate have been recorded in Table 12 while the TLC plates have been shown in Fig 1.

The qualitative analysis showed that QG contained alkaloids, amino acid, protein, glycoside, flavonoid, phenol, resin, terpene and tannin (Table 13).

Table 1 : Organoleptic Description of *Qurs-e-Ghafis*

Colour	Blackish brown
Appearance	Tablet
Texture	Hard
Taste	Bitter
Smell	Agreeable

Table 2 : Weight Variation of *Qurs-e-Ghafis*

S. No.	Weight (mg)
1	501
2	501
3	497
4	502
5	501
6	500
7	500
8	500
9	499
10	499
Mean ± SE	500.1 ± 3.37

Table 3 : Thickness and Diameter of *Qurs-e-Ghafis*

S. No.	Thickness (mm)	Diameter (mm)
1	5.40	13.25
2	5.45	13.35
3	5.50	13.30
Mean ± SE	4.50 ± 0.03	13.50 ± 0.03

Table 4 : Disintegration time of *Qurs-e-Ghafis*

Sl. No.	Disintegration time in the water (seconds)	Disintegration time in simulated gastric fluid (seconds)
1	14	11
2	16	11
3	14	13
Mean ± SE	16 ±0.57	12.33 ±0.88

Table 5 : Friability Test of *Qurs-e-Ghafis*

S. No.	Friability (%)
1	1.90
2	1.80
3	1.85
Mean ± SE	1.80 ± 0.02

Table 6 : Alcohol and water soluble contents of *Qurs-e-Ghafis*

S. No.	Alcohol Soluble Content (%)	Water Soluble Content (%)
1.	33.933	28.46
2.	34.86	15.66
3.	34.69	24.70
Mean ± SE	34.49 ± 0.28	22.94 ± 3.79

Table 7 : pH values of 1% and 10% solution of *Qurs-e-Ghafis*

S. No.	1% solution	10% solution
1.	5.80	5.35
2.	5.70	5.40
3.	4.65	5.36
Mean ± SE	5.38 ± 0.36	5.37 ± 0.01

Table 8 : Moisture content of *Qurs-e-Ghafis*

S. No.	Moisture %
1.	7.3
2.	7.8
3.	7.5
Mean ± SE	7.53± 0.14

Table 9 : Ash values of *Qurs-e-Ghafis*

S. No.	Total ash %	Acid insoluble ash %	Water soluble ash %
1.	11.94	8.01	2.12
2.	12.79	7.89	2.32
3.	12.73	8.12	1.98
Mean ± SE	12.48±0.27	8.01±0.06	2.14±0.09

Table 10 : Loss of weight on drying of *Qurs-e-Ghafis*

S. No.	Loss on drying %
1.	8.73
2.	6.8
3.	6.5
Mean ± SE	7.43± 0.69

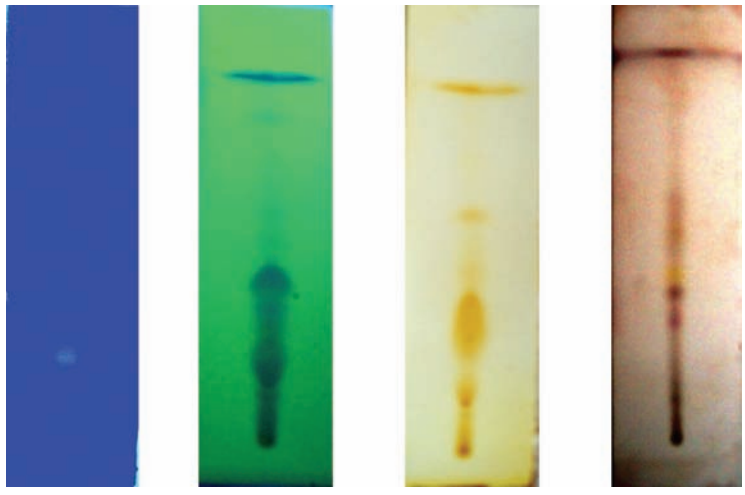
Table 11 : Successive Extractive Values of *Qurs-e-Ghafis*

S. No.	Petroleum ether %	Diethyl ether %	Chloroform %	Benzene %	Alcohol %	Water %
1	2.60	2.71	1.53	0.88	2.30	7.92
2	2.50	2.65	1.32	0.70	2.14	8.81
3	2.30	2.43	1.45	0.65	2.20	7.45
Mean ± SE	2.46 ± 0.08	2.59 ± 0.08	1.43 ± 0.06	0.74 ± 0.06	2.21 ± 0.04	8.06 ± 0.39

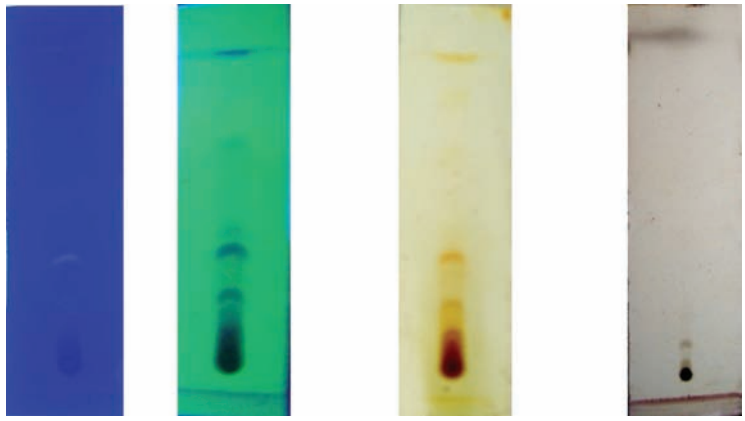
Table 12 : TLC Profile of *Qurs-e-Ghafis* extract

Extract	Solvent System	Detection / Observations		
		Visible	Number of the spots	Rf value
Chloroform	Chloroform: Benzene (4 : 1)	UV, Short	1	0.3
		UV, Long	5	0.05, 0.08, 0.18, 0.31
		Iodine	2	0.05, 0.08
		10% per chloric acid	2	0.05, 0.08

Extract	Solvent System	Detection / Observations		
		Visible	Number of the spots	Rf value
Petroleum ether	Petroleum ether : Diethyl ether (3:2)	UV short, Florescent	1	0.53
		UV, Long	5 6 6	0.10, 0.33, 0.44, 0.54, 0.61
		Iodine		0.10, 0.33, 0.44,0.54, 0.61, 0.87
		10% per chloric acid		0.11, 0.15, 0.29, 0.31, 0.44, 0.61



Petroleum ether extract



Chloroform extract

Fig. 1. TLC Profiles of *Qurs-e-Ghafis*

Table 13 : Qualitative tests for various chemical constituents in *Qurs-e-Ghafis*

Sl. No.	Test	Result
1.	Alkaloid	+ve
2.	Amino acid	+ve
3.	Protein	+ve
4.	Glycoside	+ve
5.	Flavonoid	+ve
6.	Phenol	+ve
7.	Resin	+ve
8.	Sugar (Reducing)	+ ve
9.	Sugars (Non-reducing)	+ ve
10.	Sterol/Terpene	+ve
11.	Tannin	+ve

Discussion

Physicochemical standardization is a pre-requisite in quality control of Unani drugs, both single as well as compound formulations. The efficacy of a drug mainly depends upon its physical and chemical properties therefore, the determination of physic-chemical characters to ascertain the authenticity of a drug is necessary before taking it up for pharmacological studies. The data generated in this study will help to assure the quality of this pharmacopoeal preparation.

For establishing the physicochemical standards of tablets weight variation test was conducted because a good quality tablet should be accurate and uniform in weight. The mean value of weight of ten tablets of test drug was found to be 500.1 ± 3.37 mg which is almost equal to the desired weight of 500 mg. The diameter of a tablet can vary without any change in its weight due to certain variation in procedures adopted during the processing of crude drug and shaping it to specific dosage form. The means of the diameter and thickness were found to be 13.50 ± 0.03 mm and 4.50 ± 0.03 mm, respectively, while no significant difference was observed between different samples. It has been considered desirable that after administration, the tablet should disintegrate readily as the fast dissolving tablets apart from having quick onset of effect are thought to be suitable for treatment compliance. Therefore, the tablets were subjected for the evaluation of disintegration time. The mean values of disintegration time in water and in simulated gastric fluid were found to be 16 ± 0.57 seconds and 12.33 ± 0.88 seconds, respectively. It has been reported that plain tablets / pills pass the test if each of the six plain uncoated tablets disintegrates in not more than 45 minutes (Anonymous, 1989). The test drug showed relatively less disintegration time in a medium simulating with the

gastric fluid in terms of pH, as compared to water suggesting that it can be easily and readily dissolved in the stomach leading to an early onset of effect.

Friability test is conducted to evaluate the ability of tablets to withstand abrasions under defined conditions. It is a phenomenon whereby the surface of a tablet is damaged when it is subjected to mechanical shock. A tablet should be indurate enough to withstand the attrition or shock. A loss of less than 1% however is considered acceptable by industrial standard. The mean percentage of friability in present study was found to be 1.80 ± 0.02 which is a bit more than the acceptable limit. It is warranted therefore that certain measures should be adopted to make the tablet little harder to defy the defined level of shock so as to bring the friability to 1% or less.

The extractive value is a parameter used to detect the adulteration in any drug. The amount of the extract that the drug yields in a solvent is often an approximate measure of the amount of certain constituents that the drug contains. Therefore, for establishing the standards of any drug the extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one (Jenkins *et al* ., 1967). The mean percentage of successive extractive values of QG in different organic solvents was found to be 2.46± 0.08, 2.59± 0.08, 1.43± 0.06, 0.74±0.06, 2.21± 0.04, 8.06±0.39 with petroleum ether, diethyl ether, chloroform, benzene, alcohol and water, respectively. The mean percentage of alcohol and water soluble contents by cold method were found to be 34.49 ± 0.28 and 22.94 ± 3.79, respectively. The mean percentage of the moisture content was found to be 7.53± 0.14. Ash value is the residue that remains after complete incineration of the drug. Ash value is considered an important parameter to ascertaining the standard of a drug. The dust, earthy and non-required matters are generally added in the stock of drug to increase its weight. In such a case the higher ash percentage will be found in the residue. Therefore, the ash value determination furnishes the basis of judging the identity and cleanliness of a drug and give information related to its adulteration with inorganic matter (Jenkins *et al.,* 1967). The mean percentage of the Total ash, acid-insoluble ash and water soluble ash may be a useful measure to assure the standard of QG.

Percentage of loss in weight on drying at 105^{o}C indicates the loss of volatile substances along with water which is determined by subtracting the moisture content of the drug from the loss in weight on drying. The mean percentage of loss of weight on drying found to be 7.43± 0.69 was within the normal limits. The pH value of the drug is also an important parameter of standardization of a drug. Further it also decides the kinetics of the drug when it is administered through oral route (Gilman *et al*., 2001). The mean of the pH value of 1% and

10% solution was found to be 5.38± 0.36 and 5.37± 0.01, respectively which is about two degree higher than the gastric pH. This will again help in early dissolution of the drug.

Thin layer chromatography is one of the important parameters used for detecting the adulteration for evaluating the quality of the drugs. The different kinds of chemical components are separated on TLC plate and appear in the form of spots. The calculation of R_f values after detecting the spots give an index of identity, purity and strength. If the drug is adulterated there might be the appearance of other compounds present as adulterant and in turn the number of spots may increase. On the other hand the exhausted or deteriorated drugs may lose the components and the number of spots appeared might be less. Keeping this in mind TLC studies of different extracts of test drug obtained in different organic solvents were conducted, and R_f values of various spots appeared in different solvents system were noted as a mark of identity and purity.

Qualitative phytochemical analysis of the tablet was also carried out for the determination of the presence of alkaloids, amino acids, flavonoids, phenols, proteins, resins, sterols/terpenes, sugars, glycosides and tannins. The therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents present in the drugs. The lower percentage of chemical constituents may be an important cause of less therapeutic values. Therefore, our findings recorded in this study will be helpful in predicting the biological activity of the drug.

Standardization and quality assurance of single drugs are in vague and a number of drugs of Unani medicine including the ingredients of QG have been standardized on physico-chemical and analytical parameters, but findings of single drugs cannot be extrapolated on compound drugs in order to establish their quality standard. The ingredients taking part in formation of a compound may lose at least partially, their physico-chemical properties under the influence of the operating procedures used to prepare the combination; the combination formulated may possess an entirely new entity. The present study will help to ensure and maintain the quality of QG and thereby it's biological and therapeutic effect.

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