ORIGINAL ARTICLE

Development of standard operating procedures (SOPs) and evaluation of antimicrobial activity of a polyherbal formulation *Qurṣ-i Bel*

Shayni Khan, Uzma Viquar*, Javed Inam Siddiqui, Md. Sanaul Moin, Md. Aftab Alam, Munawwar Husain Kazmi

Department of Ilmul Advia (Pharmacology), National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, Telangana, India

Abstract

Introduction: Herbal medicines are being used since ancient times. Although herbal medications are considered safe, their authenticity and purity have been questioned. It is essential to establish relevant SOPs at the source to ensure the authenticity, purity, quality, and efficacy of herbal medications. Antibiotic resistance is developing every day and is now considered a severe health hazard all over the world, so new medications from natural sources are needed. Therefore, the objective of the study was to develop the SOPs and to evaluate the antimicrobial activity of *Qurṣ-i Bel* (QB). **Materials and Methods:** In the present study, 12 batches of QB were prepared to develop SOPs with Loabe Samaghe Arabi (mucilage of *Acacia arabica*) (GAM), a 100 mesh sieve, and various temperatures and drying times employed for each batch. The antimicrobial activity was done using the agar well diffusion method against four pathogenic bacteria, that is, *Staphylococcus aureus, Escherichia coli, Klebsiella species,* and *Pseudomonas species.* **Results:** The final batch (batch no.12), in which 15% w/w GAM used as a binding agent and dried at 90°C for 120 min showed hardness 17.66 ± 0.57–18.33 ± 1.15 kg/cm^2, friability 0.0192 ± 0.00–0.0199 ± 0.00 %, and disintegration time 14.00 ± 0.00 min. The findings revealed that the methanolic and hydroalcoholic extracts of QB have antibacterial action against the three pathogens examined (*Staphylococcus aureus, Escherichia coli, and Pseudomonas species*). While, *Klebsiella pneumoniae* showed no zone of inhibition for all concentrations in all three extracts and aqueous extract was found ineffective against all tested organisms and showed no zone of inhibition. **Conclusion:** Its SOPs might be employed as traditional medicine for the treatment of various infections and could be used as a future reference for process standardization.

Key words: Antimicrobial activity, herbal, *Qurṣ-i Bel*, standard operating procedures, unani

INTRODUCTION

Remarkably, the interest in the uses of plant origin traditional medicine is growing nowadays, due to a long history of safe application recommendation and firm belief that herbs are natural and innately safe. The WHO stated around 4 billion people with 80% of the global total confidently trust traditional herbal medicine for primary health care.[1] The traditional system of medicine is being practiced in many countries of the world such as Chinese medicine (TCM), Indian system of medicine (ISM), and African folklore, where the remedies based on these are thoroughly documented and compiled.[2] Due to holistic approach and safe status of herbal Unani drugs, they are also in great demand and gaining wide acceptance globally.[3] Although herbal medicines have been used for centuries, standards and specifications must focus on basic research, such as their legitimacy and purity. Because of the complicated nature of herbal medicinal plants, as well as contamination with toxic medicinal plants and adulterants, the production and primary processing of herbal medicines have a direct impact on the quality parameters of

Address for correspondence:
Dr. Uzma Viquar, Department of Ilmul Advia (Pharmacology), National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, Telangana, India. E-mail: viquar.uzma@gmail.com

Received: 27-06-2022
Revised: 11-08-2022
Accepted: 25-08-2022

herbal medicines, so there is a need to develop or implement necessary SOPs for producing good quality Unani drugs to overcome contamination and adulteration.[4] Most of the time the Unani formulation contains different types of crude drugs which require some specific and standard manufacturing procedures.[5] If proper guidelines/SOPs of manufacturing are followed, then the substandard values in the quality of the drugs can be reduced and prepared medicine from each and everywhere uniformly. Hence, there is a need to develop SOPs of each and every formulation and should follow these developed SOPs in the future.[6]

Many natural products and their derivatives have been recognized as potent antimicrobial agents by the various researchers. In this present era, most of the microorganism developed resistance to many antibiotics which creating an immense clinical problem to treat the infectious disease.[7] Antibiotic resistance is a major health concern around the world and associated with adverse effects, therefore, there is a need to search for new drugs from novel natural sources, such as plant. At present, the resistance toward the antibiotic is increasing and that is considered a serious problem in terms of public health.[8] Respiratory tract diseases, sexually transmitted diseases, and urinary tract diseases are more likely complicated by drug resistance.[9] Unani system of medicine (USM) has holistic approach which correct the imbalance in humors and facilitate the regaining of health and sometimes considered to have anti-infective activities.[10]

Ancient Unani physicians devised the Qurṣ (Tablet) as one of the most rudimentary solid unit dosage forms. *Qurṣ-i Bel* (QB) is a popular polyherbal Unani formulation for the management of *Ishāl* (Diarrhea) and *Zaḥīr* (Dysentery). As a result, SOPs of QB for particle size, binder, drying temperature, and drying duration were produced in this work for achieving optimum friability, hardness, disintegration time, and other quality specifications. Keeping in this of the increase incidence of drug/antibiotics resistance in conventional therapy, an alternative anti-infective agent from natural drugs is the utmost need nowadays, so in the present study, antimicrobial activity of the Unani formulation QB was also evaluated.

MATERIALS AND METHODS

Ingredients of Qurṣ-i Bel

The ingredients of *Qurṣ-i Bel*, *Belgirī* (fruit pulp of *Aegle marmelos*), *Zanjabīl* (Rhizome of *Zingiber officinale*), and *Tewāj* (bark of *Holarrhena antidysenterica*) all are in equal parts.

Procurement of Raw Drugs

The formulation ingredients, that is, *Belgirī and Zanjabīl* and *Tewāj,* are procured from the GMP certified pharmacy of NRIUMSD, Hyderabad, and from registered local market/herbal dealers of Hyderabad. The ingredients were authenticated and identified by the botanist of NRIUMSD, Hyderabad, with separate voucher no. for each ingredient. The sample specimens were preserved under voucher no. SMPU/CRI-Hyd14331,SMPU/CRI-Hyd14332,SMPU/CRI-Hyd14333 for *Zanjabīl, Belgirī, and Tewāj,* respectively.

Powder Preparation to the Necessary Particle Size

All crude drugs were powdered with the help of an electric grinder and passed through sieve no. 100 to get powders of particle size of dimension less or equal to 150 µm for preparing different batches of QB [Table 1].[11]

Preparation of Binder

NFUM recommends using a variety of binders for making aqrās (tablets). In this study, Loabe (mucilage) Samaghe Arabi (*Acacia arabica*) (GAM) was selected to prepare different batches of QB. Binder GAM was taken in 10%, 12%, and 15% of the total weight of powdered drug. Mucilage or loab (binder solution) was prepared with binder: water (purified) in 4:6 w/w ratio initially [Table 1].[12]

Preparation of Lubdi (Wet massing)

Lubdi was prepared by adding binder solution to previously mixed powder drug material with suitable amount of water and then this mass was put in mixer grinder for proper mixing of binder. Precaution was taken that prepared mass should not be so hard and not so loose.

Preparation of Granules

The powdered mixture was granulated using oscillating granulation machine and passed through mesh sieve no. 20.[11]

Drying of Granules

The granules of different batches were dried at different temperatures (60, 70, 80 and 90 degrees) for different time period (60, 90 and 120 minutes) i.e., First batch was dried at 60 degree for 60 minutes, 2nd batch dried at 60 degree for 90 minutes, and so on [Table 1].[13]

Compression

The dried granules so obtained were subjected to compression by multi-station rotary presses (Tableting machine) at 6 ton pressure for all batches, and it was calibrated to the weight of tablet approximately for 500 mg/tablet.

Drying of Tablets

Prepared tablets [Figure 1] of all batches were dried in shade for 30 min for removing additional moisture, if any, and were then kept in airtight glass container.[11]

Assessment of Parameters for All Batches

All 12 batches of QB were tested 3 times for hardness, friability, and disintegration time, with the mean values serving as standard parameter values for each batch were noted.

Hardness Test

The Monsanto hardness tester was used to determine the hardness of the tablets. Three QB tablets were taken from each batch in terms of kg/cm^2, and the average values were noted.[14]

Friability Test

The friability was tested using the DBK friability test apparatus (40FTA02). Twenty QB tablets are taken from each batch and weighed in analytical balance, 10 tablets were placed in each friability tester chambers and the friabilator was placed spinning at 25 rpm for 100 rotations.[14,15]

The friability (F) is calculated by the formula:

$$F = \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 = Initial weight of tablets and W_2 = Final weight of tablets

Determination of Disintegration Time

DBK tablet disintegration test apparatus (40TDA01) was used for the determination of disintegration time. Three QB tablets were placed in the tube, which was raised in such a way where the entire up and down movement was repeated thirty times per minute. The tablets were disintegrated when no particle could pass through the gauze easily, and the time it took for the tablets to disintegrate was recorded.[14,16]

Antimicrobial Activity of Qurṣ-i Bel

Study design

Antimicrobial activity of the study formulation QB was carried out on three different extracts such methanol (ME), hydroalcoholic (HA), and aqueous (Aq) extracts. Determination of antibacterial activity and determination of minimum inhibitory concentration (MIC) of study formulation were the main objectives.

Tested Organisms and Standard

The test organisms used in this research study were consisted *Staphylococcus aureus, Escherichia coli, Klebsiella species,* and *Pseudomonas species* and the cultures of test organisms were obtained from Clinical Microbiology Laboratory, ESIC Medical College, Hyderabad. Gentamicin was used as a positive control in this research.

Preparation of Media

Mueller-Hinton agar (High media) was prepared and sterilized by autoclaving for 15 min at 15 pounds of pressure (121°C). Whole process pH was maintained between 7.2° ± 2°C at room temperature.

Preparation of Plant Extracts

A 10 g of each of fine ground powder of QB were mixed with 100 ml of methanol (High media), 100 ml of hydroalcohol (50% water and 50% ethanol), and 100 ml of distilled water separately. During the first 6 h, they were frequently shaking, and then, they were allowed to stand for 18 h. The extracts were collected after filtration using Whatman No. 1 filter paper. The filtrate was dried on water bath at temperatures below 40°C. The dried powder of formulation was dissolved in different solvents (methanol, hydro-alcohol and distilled water) at different concentrations (150, 200, 250 and 300 mg/ml i.e., each extract was dissolved in 150, 200, 250 and 300 mg/ml.[17,18]

Antimicrobial Activity Test

The antimicrobial activity of the study formulation was determined using the agar well diffusion method to detect the presence of antibacterial activity. The diameter of zones of inhibition was measured in mm and the findings were recorded to determine microbial growth.[19]

Agar Well Diffusion Method

Agar well diffusion method is a well-accepted method and from the cultures of the test organisms, about 100 µl of each of the test organisms was inoculated into already prepared Mueller-Hinton agar (High media) plates and spread immediately with the help of glass spreader and allowed to dry for 5 min. Following that, four wells were drilled in each plate using a sterilized cork borer with an 8 mm diameter. Then with the proper labeling of wells, 100 µl of the extracts of each solvent of study formulation was introduced into the wells at different concentrations of 300 mg/ml, 250 mg/ml, 200 mg/ml, and 150 mg/ml, respectively. The gentamicin disk used as a standard drug was inoculated into the middle of the four wells. They were allowed to stand for 1 h to allow for appropriate diffusion before being incubated for 24 h at 37°C.

Table 1: Description of batches of *Qurṣ-i Bel* prepared

Batch no.	Method of Preparations					
	Sieve no.	Particle size (μm)	Binder GAM (%)	Temperature of drying (°C)	Duration of drying (min)	Post-compression drying (min)
1	100	150	10	60	60	30
2	100	150	12	60	90	30
3	100	150	15	60	120	30
4	100	150	10	70	60	30
5	100	150	12	70	90	30
6	100	150	15	70	120	30
7	100	150	10	80	60	30
8	100	150	12	80	90	30
9	100	150	15	80	120	30
10	100	150	10	90	60	30
11	100	150	12	90	90	30
12	100	150	15	90	120	30

A distinct zone of inhibition surrounding the wells revealed the test organism's sensitivity to methanol, hydroalcohol, and water extracts of QB. Plates were examined for zone of inhibition after incubation, and the diameter of the clear zone (zone of inhibition) was measured using a transparent ruler to the closest millimeter. The experiment was repeated 3 times, and the inhibition zones were recorded as mean standard error.[19,20]

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) for the test organisms was determined in triplicates for each at different concentrations of 300, 250, 200, and 150 mg/ml. A 100 μl of the test organism was placed into pre-diluted 0.5 McFarland turbidity standard tubes after 1 ml of nutritional broth was added. To act as a control, a tube containing simply nutritional broth was inserted with the test organism. After a 24 h incubation period at 37°C, all of the tubes were inspected for growth by looking for turbidity.[21]

Statistical Analysis

All measurements were performed in triplicate. The data were expressed as mean ± SD. About 95% level of significance ($P \leq 0.05$) was used for the statistical analysis.

RESULTS

SOPs for manufacturing process of QB were developed by assessing each batch 3 times for hardness, friability, and disintegration time and mean regarded as standard parameter value was also noted. The results of all batches

Figure 1: Formulation "*Qurṣ-i Bel*"

are given in Table 2. The batch no. 12 was selected as a final batch as it showed most appropriate result among all the batches with particle size 150 μm (100 mesh sieve), in which 15% w/w GAM used as a binder and dried at 90°C for 120 min. The mean value of hardness of final batch was found to be 17.66 ± 0.57–18.33 ± 1.15 kg/cm^2, friability was found to be 0.0192 ± 0.00–0.0199 ± 0.00%, and disintegration time was found to be 14.00 ± 0.00 min and all of its conditions regarding particle size, binder, temperature, and duration of drying were considered as its SOPs. The obtained data of final batch (batch no. 12) are represented in Figures 2-4 for hardness, friability, and disintegration time, respectively.

Table 3 and Figure 5 show the result of final batch, obtained from the antimicrobial activity of QB. The result showed that *Staphylococcus aureus* showed effective zone of inhibition of 12.66 ± 0.57–18 ± 1.00 and 11.66 ± 1.52–17.33 ± 1.15 (mean ± SD) in methanolic and hydroalcoholic extracts, respectively.

Table 2: Results of all batches of *Qurṣ-i Bel*

Batch no.	Method of preparation					Hardness (kg/cm²)	Friability (%)	Disintegration time (minutes)
	Sieve no.	Particle size (µm)	Binder GAM (%)	Temperature of drying (°C)	Duration of drying (min)	Mean±SD	Mean±SD	Mean±SD
1	100	150	10%	60	60	5.20±0.52–5.33±0.56	1.5232±0.52–1.7449±0.58	7.00±0.54–8.15±0.43
2	100	150	12%	60	90	8.52±0.42–9.65±0.55	1.2462±0.45–1.4469±0.78	11.15±0.54–12.35±0.43
3	100	150	15%	60	120	12.26±0.50–13.43±0.92	0.0482±0.68–0.0589±0.53	13.82±0.52
4	100	150	10%	70	60	5.20±0.52–5.33±0.56	1.5232±0.52–1.7449±0.58	7.00±0.54–8.15±0.43
5	100	150	12%	70	90	8.52±0.42–9.65±0.55	1.2462±0.45–1.4469±0.78	11.15±0.54–12.35±0.43
6	100	150	15%	70	120	14.56±1.12–15.68±0.87	0.0342±0.46–0.0429±0.54	13.25±0.45
7	100	150	10%	80	60	5.20±0.52–5.33±0.56	1.5232±0.52–1.7449±0.58	7.00±0.54–8.15±0.43
8	100	150	12%	80	90	8.52±0.42–9.65±0.55	1.2462±0.45–1.4469±0.78	11.15±0.54–12.35±0.43
9	100	150	15%	80	120	15.76±0.58–16.65±0.78	0.0392±0.00–0.0419±0.00	13.14±0.25
10	100	150	10%	90	60	5.20±0.52–5.33±0.56	1.5232±0.52–1.7449±0.58	7.00±0.54–8.15±0.43
11	100	150	12%	90	90	8.52±0.42–9.65±0.55	1.2462±0.45–1.4469±0.78	11.15±0.54–12.35±0.43
12	100	150	15%	90	120	17.66±0.57–18.33±1.15	0.0192±0.00–0.0199±0.00	14.00±0.00

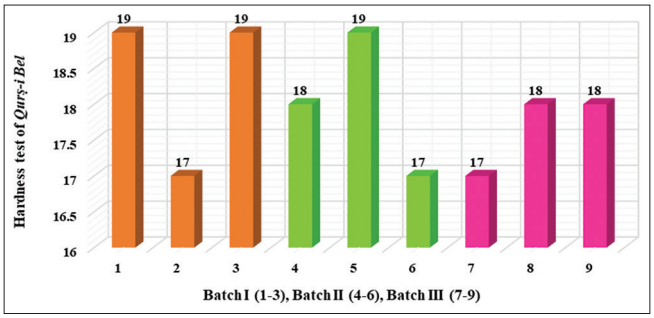

Figure 2: Hardness test of *Qurṣ-i Bel*

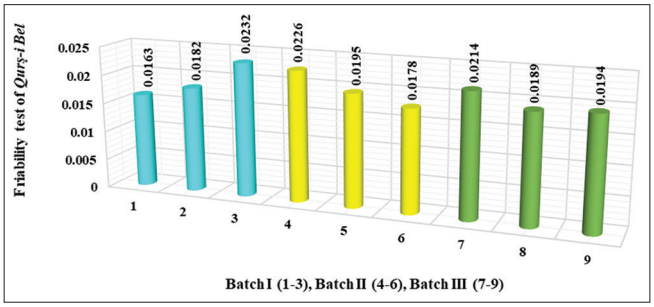

Figure 3: Friability test of *Qurṣ-i Bel*

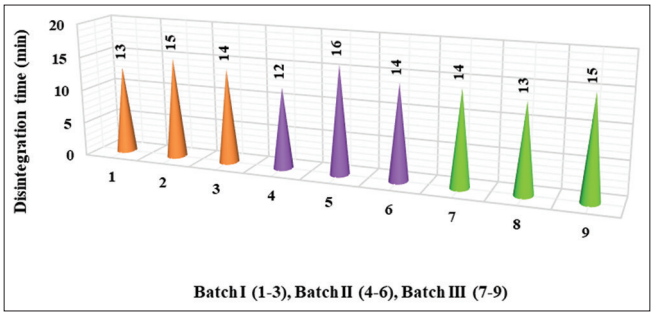

Figure 4: Disintegration time of *Qurṣ-I Bel* in aqueous media (min)

Escherichia coli showed effective zone of inhibition of 11.66 ± 0.57–17 ± 1.00 and 11.33 ± 1.52–15.66 ± 1.15 (mean ± SD) in methanolic and hydroalcoholic extracts, respectively. *Pseudomonas aeruginosa* showed effective zone of inhibition of 11.33 ± 1.52–14 ± 1.00 and 11 ± 1.00–13.33 ± 0.57(mean ± SD) in methanolic and hydroalcoholic extracts, respectively. *Klebsiella pneumoniae* showed no zone of inhibition for all concentrations in all three extracts and aqueous extract was found ineffective against all tested organisms and showed no zone of inhibition.

Table 3: Effect of *Qurṣ-i Bel* extracts on tested organisms as zone of inhibition (mm)

Microorganisms	Extracts	Zone of inhibition in mm (Mean±SD)				
		150 mg/ml	200 mg/ml	250 mg/ml	300 mg/ml	Standard drug
Staphylococcus aureus	ME	12.6±0.57	14.6±0.57	15.3±0.57	18±1.00	21±0.00
	HA	12.3±1.15	11.6±1.52	13.3±1.52	17.3±1.15	
	Aq	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	
Escherichia coli	ME	11.6±0.57	13±1.00	14.3±0.57	17±1.00	17±0.00
	HA	11.3±1.52	13.6±1.52	14±1.00	15.6±1.15	
	Aq	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	
Klebsiella pneumoniae	ME	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	16±0.00
	HA	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	
	Aq	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	
Pseudomonas aeruginosa	ME	11.3±1.52	12±1.00	13.6±0.57	14±1.00	19±0.00
	HA	0.0±0.00	11±1.00	12.3±1.52	13.3±0.57	
	Aq	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	

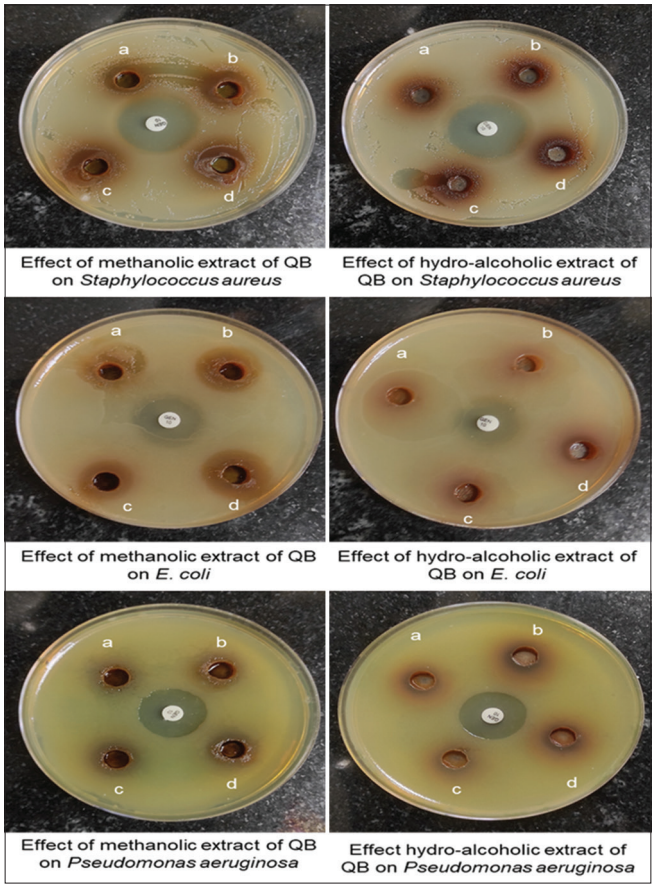

Figure 5: Zone of inhibition (mm) of *Qurṣ-i Bel* extracts on tested organisms. (a – 150 mg/ml, b – 200 mg/ml, c – 250 mg/ml, and d – 300 mg/ml)

Table 4 shows the result of minimum inhibitory concentration (MIC). The result indicated that methanolic extract of the QB formulation showed a minimum inhibitory concentration (MIC) of 150 mg/ml against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* Whereas, the hydroalcoholic extract of QB formulation showed a minimum inhibitory concentration (MIC) of 200 mg/ml against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and a minimum inhibitory concentration (MIC) of 150 mg/ml against *Escherichia coli* was observed.

DISCUSSION

The Unani system of medicine is one of the well-recognized Indian Systems of Medicine and keeps a respectable position in health-care system, but Unani medicines have been criticized for its poor quality prospective and presentation.[22] The development of SOPs is an essential tool to assure the quality of medicines. Process of manufacturing to develop standard operating procedures of QB starts from authenticity of crude drugs. The good quality of tablet depends on the particle size, binder, time of drying, and temperature of drying. Particle size plays an important role in dissolution of drug and the rate of dissolution is directly proportional to the surface area of the drug.[23] An attempt has been made to maintain the uniform particle size in respect of sieve no. Binder, temperature, and duration of drying also play an important role in friability, hardness, and disintegration of tablets. Binder is added as solution in the powder for preparation of granules as they are more effective with appropriate solution form. Drying and temperature are most commonly used in pharmaceutical manufacturing process in the preparation of different dosage forms and helps in preservation of drugs by minimizing the penetration for mold and bacterial growth. Post-compression drying was done to further overcome the moisture and contamination issue.[24] The hardness and friability are important parameters for determining the mechanical strength of tablets. These two estimations are very useful for tablet quality control in tablet manufacturing to avoid fracture and breakage during handling, displacement, and transportation. Tablet requires a certain amount of strength or hardness to withstand

Table 4: Effect of *Qurṣ-i Bel* extracts on tested organisms as minimum inhibitory concentration **(MIC)**

Tested organisms	MIC (mg/ml)		
	Methanolic extract	Hydroalcoholic extract	Aqueous extract
Staphylococcus aureus	150	200	ND
Escherichia coli	150	150	ND
Klebsiella pneumoniae	ND	ND	ND
Pseudomonas aeruginosa	150	200	ND

mechanical shocks in manufacturing, packaging, and shipping. As such if the tablet is too soft, it will not be able to withstand handling during post-processing. Moreover, if the tablet is too hard, it will not disintegrate in the required period of time.[25] Friability is also another important parameter of tablet's strength and it should be <1%. Disintegration test is used to determine whether tablet or pill disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions.[26] This test is very useful as a quality assurance tool for conventional dosage forms. The tablets have a minimum disintegration time of 5 min and maximum disintegration time of 30 min.[24] Hence, keeping these points in mind, 12 batches were decided to prepare using different concentration of binder (GAM), that is,10%, 12%, and 15% of the total weight of powdered drug and dried in hot air oven in different variables of temperature and duration. The granules of different batches were dried at different temperatures (60, 70, 80 and 90 degrees) for different time period (60, 90 and 120 minutes) in hot air oven and batches were finally selected based on the hardness, friability and disintegration time. Out of these, batch no. 12 was selected as final batch on the basis of lowest friability, optimum greater hardness, and good and appropriate disintegration time. Decrease in friability was noted on increase the concentration of binder in different batches, an increase in binder concentration also increases the hardness, and it was also observed that increasing in drying time, that is, up to 120 min also increase the hardness, efforts were made to maintain hardness to improve the strength of tablet as lesser hardness and excess friability is commonly encountered in Unani tablets. Finally, the SOPs of *Qurṣ-i Bel* were developed and it was found that the powdered crude drug passed through 100 mesh sieve and mixed with 15% Loabe Samaghe Arabi (mucilage of gum acacia) as binder and dried at 90°C for 120 min.

Since ancient times, plants have been a veritable source of drugs. However, people tend to ignore the importance of herbal medicine. Various researchers revealed that the physiological and medicinal properties of herbal products are due to the presence of phytochemical biologically active compounds. The antimicrobial activity of a drug is due to specific phytochemicals or essential oils. The main factors that determine the antimicrobial activity are the composition of the drug, amount used, type of microorganism, pH value, and temperature of the environment.[27,28] Many researchers have reported on the antimicrobial properties of every ingredient of QB. Hence, this research work was undergone to evaluate the antimicrobial activity of methanolic, hydroalcoholic, and aqueous extracts of QB. The methanolic, hydroalcoholic, and aqueous extracts of QB were subjected to an antimicrobial activity test using the agar well diffusion method against four pathogenic bacteria, that is, *Staphylococcus aureus, Escherichia coli, Klebsiella species,* and *Pseudomonas species* and the effects were compared with commonly used antibacterial agents (gentamicin). The results of this study showed that significant zone of inhibition (in mm) was observed on three bacteria *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas species* whereas, *Klebsiella pneumoniae* showed no zone of inhibition for all concentrations in all three extracts. During the present work, the methanolic extract was found as most effective, it could be as a result of better extraction with methanol solvent and the highest antibacterial activity was observed against *Staphylococcus aureus* with effective zone of inhibition of 12.66 ± 0.57–18 ± 1.00 (mean ± SD). *Staphylococcus aureus* and *Escherichia coli* were highly susceptible to the methanolic and hydroalcoholic extracts of QB. *Pseudomonas species* was highly susceptible to the methanolic extract but relatively resistant to the hydroalcoholic extract of QB as there was no inhibition at 150 mg/ml. *Klebsiella species* was completely resistant to all extracts of QB and there was no inhibition even at the highest concentrations. While, aqueous extract was found ineffective against all tested organisms and showed no zone of inhibition, which showed in Table 3 and Figure 5. Their potency was assessed by MIC value which showed in Table 4. Hence, the present study states that the antimicrobial activity of QB is dependent on the concentration and the solvent of extraction. Different organic and inorganic solvents exist for the extraction of plants and studies have showed over the years that organic extraction is more active than aqueous extraction.

CONCLUSION

The present study was undertaken to develop the standard operating procedures (SOPs) and to evaluate the antimicrobial activity of polyherbal Unani formulation, *Qurṣ-i Bel*. It may be concluded that the approach utilized to make the final batch, that is, 150 µm particle size (100 mesh sieve), 15% w/w GAM employed as a binder, dried at 90°C for 120 min, and can be used as SOPs for future process standardization references. In the present study, the antimicrobial effect was also evaluated and the study drug, QB showed the

antibacterial activity against the tested organisms. Hence, in the preliminary way, it can be used as antibacterial agent and there is a need to further enhance its exploitation in this regard. The findings of this study have offered evidence for QB's therapeutic potential and showed that two types of extracts (methanolic and hydroalcoholic) of QB possess potential antibacterial activity. In the future, furthermore, screening test and studies should be conducted for using QB as a medicine for treating other infections and various diseases. Moreover, with the developed quality standards of QB, the drug can be reproducible and to maintain the quality assurance in the future.

ACKNOWLEDGMENT

The authors would like to express their gratitude to Prof. Asim Ali Khan, Director General, CCRUM, New Delhi, and Dr. Ahmed Minhajuddin, Director In-charge, NRIUMSD, Hyderabad, for providing the essential facilities and infrastructure.

REFERENCES

1. Ara SA, Viquar U, Zakir M, Urooj M, Kazmi MH, Husain GM. Preclinical toxicity evaluation of Habb-e-Azaraqi: A nux-vomica-based traditional Unani polyherbal formulation in rats. Tradit Kampo Med 2021;8:29-41.
2. Bijauliya RK, Alok S, Chanchal DK, Kumar M. A comprehensive review on standardization of herbal drugs. Int J Pharm Sci Res 2017;8:3663-77.
3. Siddiqui ZA, Ansari ZA, Jabeen A, Anwar M, Zakir M, Siddiqui A, *et al*. Standard operational procedures (SOPs) and physicochemical standardisation of a classical Unani formulation-Qurs Mafāsil Jadīd. Int J Pharm Res 2021;13:2422-30.
4. Ali A, Sumbul S, Ahmad MM, Ahmad S, Kabir H, Abdin MZ. Development of standard operating procedure and standardization of Habb-e-BanafshaQawi-A Unani polyherbal formulation. J Pharm Bioallied Sci 2015;7:250-3.
5. Ali W, Uddin H, Ali A. Standard manufacturing procedure of Qurse Tabasheer-a herbo mineral Unani antidiabetic formulation. J Res Unani Med 2015;4:51-64.
6. Kumar V, Kushwaha V, Charde V, Jagtap C, Gandhi Y, Grewal J, *et al*. The validated pharmaceutical standard operating procedure and quality control study of the coded polyherbal tablet formulation AYUSH SG-5. S Afr J Bot 2022;150:1-7.
7. Aghazadeh M, Bialvaei AZ, Aghazadeh M, Kabiri F, Saliani N, Yousefi M, *et al*. Survey of the antibiofilm and antimicrobial effects of *Zingiber officinale* (*in vitro* study). Jundishapur J Microbiol 2016;9:e30167.
8. World Health Organization. Antimicrobial Resistance. Geneva: World Health Organization; 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance [Last accessed on 2022 Mar 12].
9. World Health Organization. News Release: New Report Calls for Urgent Action to Avert Antimicrobial Resistance Crisis. New York: World Health Organization; 2019. Available from: https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis [Last accessed on 2022 Mar 12].
10. Ministry of AYUSH. Unani System of Medicine: The Science of Health and Healing. Vol. 21. New Delhi: Ministry of AYUSH, Government of India; 2016. p. 1-2.
11. Anonymous. National Formulary of Unani Medicine. Part. 2. Vol. 1. New Delhi: Central Council for Research in Unani Medicine, Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 2007. p.7.
12. Waring EJ. Pharmacopeia of India. New Delhi: Asiatic Publishing House; 2005. p. 62.
13. Ali W, Shaikh H, Ansari A, Khanam S. Standardization of Unani antidiabetic tablet-Qurse Tabasheer. Pharmacognosy Res 2016;8:147-52.
14. Lachman L, Liberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. New Delhi: CBS Publishers and Distributors Pvt. Ltd.; 2013. p. 479-92.
15. Evan WC. Quality control. In: Trease and Evans Pharmacognosy. 16th ed., Ch. 16. London: Elsevier Ltd.; 2009. p. 122-6.
16. Markl D, Zeitler JA. A review of disintegration mechanisms and measurement techniques. Pharm Res 2017;34:890-917.
17. Manandhar S, Luitel S, Dahal RK. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med 2019;2019:1895340.
18. Bouyahya A, Abrini J, El-Baabou A, Bakri Y, Dakka N. Determination of phenol content and antibacterial activity of five medicinal plants ethanolic extracts from North-West of Morocco. J Plant Pathol Microbiol 2016;7:4.
19. Gupta D, Dubey J, Kumar M. Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms. Asian Pac J Trop Dis 2016;6:15-20.
20. Mirtaghi SM, Torbati Nejad P, Mazandarani M, Livani F, Bagheri H. Evaluation of antibacterial activity of *Urtica dioica* L. leaf ethanolic extract using agar well diffusion and disc diffusion methods. Med Lab J 2016;10:15-21.
21. Lucky E, Igbinosa OE, Jonahan I. Antimicrobial activity of *Zingiber officinale* against multidrug resistant microbial isolates. Health Sci Res 2017;4:76-81.
22. Mir IA, Jahan N, Sofi G, Mehfooz S, Husain M. Role of Unani system of medicine in global health care: An emerging field. Orthop Muscular Syst Curr Res 2017;6:8-10.
23. Troy DB. Oral solid dosage form. In: Remington: The Science and Practice of Pharmacy. 21st ed., Ch. 35, 45.

Philadelphia, PA: Lippincott Williams and Wilkins; 2006. p. 675, 916-8.
24. Lachman L, Lieberman HA, Kanig JL. Basic chemical principles related to suspension and emulsion dosage form. In: The Theory and Practice of Industrial Pharmacy. 3rd ed., Ch. 5, 9. Bombay: Varghese Publishing House; 1987. p. 100-21, 197-239, 293-372.
25. Anonymous. Operating Procedure for Tablet Hardness Testers (Monsanto). Pharmaceutical Guidance; 2016. Available from: https://www.pharmaguidances.com/operating-procedure-for-tablet-hardness-tester-monsanto/#:~:text=Place%20the%20sample%20tablet%20in%20the%20vertically%20holding,breakage%20of%20tablet%20shows%20hardness%20on%20the%20scale [Last accessed on 2022 Mar 12].
26. Anonymous. The United States Pharmacopoeia (USP 32) NF 27. Vol. 1. Washington, DC: The USP Convention, Rockville; 2009. p. 262.
27. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71-9.
28. Shah PH, Mamtha MT, Bhatt V, Zatakiya D, Shah SH, Gandhi B. Effect of antimicrobial activity of herbal medicines on *Streptococcus mutans*. Natl J Integr Res Med 2019;10:16-8.

Source of Support: This research was funded by Central Council for Research in Unani Medicine (CCRUM). **Conflicts of Interest:** None declared.