ABSTRACT
Background: Quality control of Unani poly herbal formulations is the need of the day for better acceptance of Unani medicine. Qurse Tabasheer (QT) is a Unani poly herbal formulation containing six ingredients, Tabasheer (Siliceous concretions) (Bambosa arundinacea Retz.), Gulnar (Punica granatum Linn. flower), Tukhme kahu (Lactuca sativa Linn. seed), Tukhme khurfa (Portulaca oleracea Linn. seed), and Gile Armani (bole) widely used in treatment of diabetes. The present study was taken up to scientifically evaluate the various physicochemical parameters to standardize the formulation. Objective: To evaluate various physicochemical parameters including ash values, moisture content, extractive values, thin layer chromatography (TLC) and high-performance TLC (HPTLC), friability, disintegration, uniformity, and weight variation for standardization of QT. Materials and Methods: Ingredients were identified by the experts. The method mentioned in national formulary of Unani Medicine with modification was followed for preparation of the tablets. Physicochemical standards were established for ideal batch of tablets on the basis of set parameters regarding friability, hardness, and disintegration. Various parameters such as organoleptic characters, extractive values for the extract and HPTLC fingerprinting postcompression were carried out for evaluation of QT. Results: Parameters for loss of weight on drying, pH, ash values, extractive values documented. Qualitative chemical tests indicated the presence of alkaloid, glycoside, tannins, and steroids. TLC and HPTLC fingerprinting studies showing the presence of major peaks were documented. Friability, hardness, and disintegration time of ideal batch was found to be within the set limit. Quality control results were within the set parameters regarding Friability, hardness, and disintegration time of ideal batch. Total fungal and bacterial counts were found to be within the limit. Conclusion: Standards were established for poly herbal formulation QT, which may be used as reference for preparation and standardization of QT. Key words: Physicochemical, Qurse Tabasheer, standardization, tablet, Unani

SUMMARY
In this work standardization of antidiabetic tablet Qurse Tabasheer with diverse ingredients including herbal and mineral origin drugs has been attempted with identification of its ingredients, formulation, physicochemical evaluation and HPTLC fingerprinting, which may help in preparing consistent and better efficacious formulations.

INTRODUCTION
Like other contemporary traditional medicine pharmacy, Unani pharmacy is also facing, several issues related to quality control of formulations such as improper use and un-sustained availability of original raw material, in efficient processing techniques leading to poor quality product, poor quality control procedure, lack of implementation of current good manufacturing practices, difficulty in maintaining batch to batch consistency etc. To overcome this, it is necessary to carry out standardization work. WHO has emphasized the need to ensure quality using modern analytical techniques and setting up physicochemical standards. Out of several oral unit dosage forms, Qurs (tablet) is one of the most suitable/practical dosage forms due to its easy portability for prolong use, stability and accuracy of dose, etc. Therefore, in the present study, physicochemical parameters for Qurse Tabasheer (QT) were investigated. Formulation selected for study is being commonly used and manufactured by the Unani pharmacies. It contains six ingredients, Tabasheer (Siliceous concretions) (Bambosa arundinacea Retz.), Gulnar (Rosa damascena Mill. flower), Gulnar (Punica granatum Linn. flower), Tukhme kahu (Lactuca sativa Linn. seed), Tukhme khurfa (Portulaca oleracea Linn. seeds), and Gile

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Cite this article as: Ali W, Shaikh H, Abdullah A, Khanam S. Standardization of unani antidiabetic tablet - Qurse Tabasheer. Phcog Res 2016;8:147-52.
This particular formulation is mentioned in Bayazee Kabeer and Kitabul Murakkabat Al Maroof Makhtizanul Murakkabat. It is used in the treatment of Dhayabitus (diabetes), Hummae Hadda (acute fever), and Ihal (diarrhoea). Moreover, this formulation has been reported for its pharmacological activity as a hypoglycaemic and there is a need to develop its quality control standards.

**MATERIALS AND METHODS**

**Collection and identification of drugs**

Gulnar, Gule Surkh, Tukhme Khurfa, and Tukhme Kahu were procured from A.B. General Store, Avenue Road; Bengaluru and identified by expert at FRLHT (Foundation for Revitalization of Local Health Traditions) Bengaluru. Tabasheer was procured from a raw drug dealer “Herbo World Associates,” New Delhi, and identified by expert. Different samples of Gile Armani were collected from crude drug market of Bengaluru, Delhi, and Malegaon (MS) and for its identification, X-ray diffraction was conducted at Department of Material Engineering, Indian Institute of Sciences Bengaluru out of which one sample was selected which was looking like of natural combination containing Fe₂O₃ Hematite; Silica (SiO₂)-Quarts alpha; CaCo₃ Calcite form and TiO₂ Titanium Oxide, Anatase) and its constituents resembled Red Ochre (Gaïrika/Geru) as per its constituent mention in Ayurvedic Pharmacopeia. The sample of Gile Armani was identified as Geru which is a genuine substitute of Gile Armani in Unani text as literature also reveals that Gile Armani (Armenian bole) is generally unavailable. This sample of Gile Armani was taken for study as ingredient of the formulation as available market sample. The drug samples were submitted in NIUM Drug Museum and voucher specimen No. 22/IS/Res/2014 was collected for future reference.

**Method of preparation of Qurse Tabasheer**

The method mentioned in National Formulary of Unani Medicine was followed for the preparation of Qurse Tabahsheer with modifications. Eighteen different batches were prepared (trial and error) by varying the following parameters: (a) Mesh size of powder (#80, 100 and 120), (b) concentration of binder (10, 15 and 20% of gum acacia), (c) duration of drying of granules (30 and 60 min at 60°C temperature), and (d) postcompression drying (30 min at 60°C).

One percentage liquid paraffin as lubricant and 1% magnesium carbonates as glidant were added slowly in dried granules. One percentage liquid paraffin as lubricant and 1% magnesium carbonates as glidant were added slowly in dried granules. Oscillating granulation machine GMP model (Cemach machinery Ltd., Ahmadabad SN. 1417) was used for granulation, Hot air oven (Labline Mod. No. HO 6.7) was used for drying and Tablet compression was done using multi-station rotary presses (tabletting machine) GMP model (Cemach machinery Ltd., Mod. No. CM-D-20).

The batch with powder #100 mesh size, binder 20% of total wt. of powder (16% in the formulation), duration of drying of granules 60 min and post compression drying for 30 min at 60°C was selected as the final batch for physicochemical standardization on the basis of set parameter, i.e. minimum friability (<1%), hardness near to standard value (4 kg) and disintegration time <30 min. Pre-compression parameters including bulk density, tapped density, compressibility index, Hausner’s ratio, and angle of repose of final batch was also done.

**Physicochemical parameters**

**Organoleptic properties**

Appearance, color, smell, and taste were evaluated.

**Friability test**

Friability test apparatus Roche’s friabilator (Labinda mod. no. 1020) was used for determination of friability of tablet. This device subjected the tablet to the combined effect of abrasion and shock in a public chamber and dropping the tablets at a height of 6 inches in each revolution. Weighed tablets were placed in friabilator revolving at 25 rpm for 100 revolutions. Tablet was de-dusted using a soft muslin cloth and weighed. F = (W₁ – W₂/W₁) × 100 (W₁ = Initial weight of tablets, W₂ = Final weight of tablets)

**Tablet hardness test**

Randomly three tablets were pickup and they were individually tested for the hardness by Monsanto hardness tester (Shital scientific industries Sr. no. 11012010) in terms of kg/cm.

**Disintegration test**

Disintegration testing apparatus (Thermonik: Mod. no. TD 20S) was used for determination of disintegration time.

**Uniformity of diameter**

Diameter of three randomly selected tablets was measured individually using a Vernier Caliper (UTTAR, IME type 6 inch/15 cm) and expressed in mm.

**Extractive value (Soxhlet apparatus)**

**Successive extractive value**

The coarse powder of QT was extracted successively using soxhlet apparatus with different solvent in increasing order of polarity, petroleum ether → benzene → chloroform → ethanol. 10 g powdered drug was taken and subjected to successive extraction with each solvent for 6 h. The extracts were filtered using filter paper (Whatman No. 1) and dried on water bath. The extractive values were determined with reference to the weight of the drug taken (w/w). The procedure was repeated 3 times to calculate mean extractive values.

**Non successive extractive value**

The coarse powder of QT was extracted separately in different solvent (water, ethyl alcohol and petroleum ether) using soxhlet apparatus. 10 g powdered drug was taken and subjected to separate extraction with each solvent. The extracts were filtered using filter paper (Whatman No. 1) and evaporate on water bath. Extractive values were determined with reference to drug taken (w/w).

**Extractive value (Cold maceration)**

Determination of alcohol and water-soluble extractive was done as per protocol for testing of Ayurvedic, Siddha and Unani Medicines.

**Ash value**

Total ash and water soluble ash were done by method mentioned in protocol for testing. Acid insoluble ash and sulphated ash were done by method mentioned in UPI.

**Loss of weight on drying at 105°C**

Loss of weight on drying (LOD) at 105°C was done by method mention in UPI.

**pH value**

pH value of 1% solution and pH value of 10% solution was determined as per the method mentioned in physicochemical standardization of Unani Medicine part IV.

**Weight variation**

Twenty tablets were selected randomly from selected batch and weighed individually. Average weight was calculated, and individual weights were
compared to average weight. If not more than 2 tablets are outside the percentage limit, tablets meet the USP test (USP weight variation test).[11]

**High-performance thin layer chromatography analysis**

**Preparation of extract of the drug for high-performance thin layer chromatography analysis**

Successive extracts (Petroleum ether, chloroform, ethanol, methanol and water) obtained by soxhlet extraction were dried and diluted with small amount of methanol, filtered by Whatman no. 41. Filter paper. The solution so obtained was used as sample for high-performance thin layer chromatography (HPTLC) analysis. "CAMAG thin layer chromatography (TLC) scanner 3" system was used for analysis along with automatic TLC applicator and UV visible cabinet as imaging system, the instrument had "win CATS-version 1.3.3" software for documentation.[9]

**Optimization of thin layer chromatography system**

TLC procedure was optimized using various solvents. The solvent system toluene: ethyl acetate: formic acid (5:4:1) which gave good resolution was selected. The samples were spotted on Merck Silica gel 60 F 254 plates (10 cm × 10 cm) using a linomat. The chamber was saturated for 10 min, and the plates were developed up to migration distance of 93 mm. Scanning wavelengths: UV 366 nm, 254 nm and 280 nm were used for visualization.

**Development of high performance thin layer chromatography technique**

After developing, TLC plates were dried completely and scanned with the CAMAG TLC scanner 3’ at 200–400 nm. λ max was found to be 280 nm. The plates were again scanned at 366, 254 and 280 nm. The number of peaks were noted and tabulated to be used as finger print.

**Qualitative test for chemical constituent**

The extracts were tested for tannin,[23] terpenoids,[23] Glycoside, alkaloids (Dragendorff’s test), protein (Millon’s test), carbohydrates (Fehling’s test), steroids (Salkowski reaction), and resins using the methods mentioned in Physiochemical Standardization of Unani formulation Part I.[24] Test for saponins,[23] flavonoids, phenols (Ferric chloride test),[23] and test for reducing sugar were also performed.[23]

Total fungal and specific pathogen like *Escherichia coli*, *Salmonella* spp., *S. aureus*, and *Pseudomonas aeruginosa* tests were done at Bengaluru test house Bengaluru by method mention in the Ayurvedic pharmacopoeia of India. Part II, Vol. 2nd ed. 1st.[25]

**RESULTS**

Organoleptic properties: Appearance: Circular uncoated tablet (slightly biconvex); Colour: Dark Brown; Smell: Rosy; Taste: Clayey, astringent and slightly bitter; Texture: Hard and smooth [Figure 1].

The mean values of precompression parameters of granules of selected batch: Bulk density (g/ml): 0.5084 ± 0.0, tapped density (g/ml): 0.5884 ± 0.006669, compressibility index (%): 13.56 ± 0.9786 and Hausner’s ratio: 1.157 ± 0.01311 and angle of repose (θ): 29.98°C. The mean value of friability (%), hardness (kg/cm), disintegration time (minutes) and diameter (mm) of QT. Were determined and the values are depicted in [Table 1].

The mean percentages of the successive and nonsuccessive extractive values were calculated, and the results are depicted in [Table 2]. The values of the alcohol and water-soluble content, total ash, water soluble ash, acid insoluble ash, sulfated ash, LOD at 105°C, pH at 1% and 10% solution were determined, and the values in mean percentage are depicted in [Table 3].

The mean value of weight of randomly selected 20 tablets was found to be 793.7 ± 4.755 mg. The deviation of individual tablet weight from the average weight of 20 tablets was found within the percentage limit of 5% of mean weight.

TLC analysis was carried out using toluene: ethyl acetate: formic acid (5:4:1) as mobile phase. Numbers of peaks in all the extracts are shown in [Table 4]. The TLC analysis of chloroform extract of the tablets showed

| Parameters                      | Mean±SEM         |
|---------------------------------|------------------|
| Friability (%)                  | 0.09±0.0057      |
| Hardness (kg/cm)                | 4.03±0.087       |
| Disintegration time (min)       |                  |
| Aquous media                    | 25.57±0.486      |
| Simulated gastric fluid (water with 0.1 M hydrochloric acid) | 24.72±0.1881 |
| Uniformity of diameter (mm)     | 13±00            |

SEM: Standard error of mean

| Solvents                      | Mean±SEM (%)     |
|-------------------------------|------------------|
| Petroleum ether               | 7.380±0.2884     |
| Benzene                       | 1.000±0.02082    |
| Chloroform                    | 0.4267±0.05239   |
| Ethyl alcohol                 | 10.93±0.3187     |
| Water                         | -                |

SEM: Standard error of mean

| Physiochemical parameters of Qurse Tabasheer | Mean±SEM         |
|---------------------------------------------|------------------|
| Alcohol soluble matter (%)                  | 9.180±0.6350     |
| Water soluble matter (%)                    | 17.08±0.6021     |
| Total ash (%)                               | 26.50±0.07638    |
| Water soluble ash (%)                       | 0.8667±0.07265   |
| Acid insoluble ash (%)                      | 21.28±0.3632     |
| Sulfated ash (%)                            | 25.85±0.2754     |
| Loss of weight on drying (105°C) (%)        | 6.027±0.1641     |
| pH value at (%)                             |                  |
| 1                                           | 5.450±0.08021    |
| 10                                          | 4.727±0.02404    |
Preliminary phytochemical studies of QT showed the presence of glycoside, tannin, terpenoid, saponins, flavonoids, alkaldoids, phenols, steroids, protein, and carbohydrates whereas steroid, reducing sugar, and resins were absent.

Total fungal and total bacterial count/g were within limit and specific pathogen such as E. coli, Salmonella spp., S. aureus, P. aeruginosa were found to be absent [Table 8].

**DISCUSSION**

Selected formulation QT has got very important indication in diabetes and is also a commonly marketed formulation for diabetes, but its standardization has not been reported, hence in the present work, an attempt has been made. Appearance, color, and texture play an important role such as in quick identification. Appearance, color, smell, taste, and texture of tablets prepared as per the standard protocol were found acceptable. The presence of a particular smell/odor could be characteristics of a drug and indicates quality and identity of particular drug. QT shows dark brown color, and a particular taste and smell, dominant smell is of Gulab (Gule surkh) and Anar (Guinar) flower. Gulab (Rose) smell was dominating over Anar (Pomegranate). Slight bitterness in taste might be due of Takhme Kahu Seeds (presence of bitter principles lectucin, lectopirin, and lactucic acid) and slightly due to Takhme khurfa. The tablet was of hard and smooth texture.

Friability was found to be within acceptable limit, i.e. 0.5–1%. It is an important parameter to measure the strength of tablets. Hardness was found to be 4.03 ± 0.087 kg/cm. It was within acceptable limit of 4 kg.

The mean percentage of the successive extractive values was found maximum in alcohol (10.93 ± 0.32). The mean percentage of the nonsuccessive extractive values was found maximum in water (27.67 ± 0.5783) followed by ethyl alcohol (13.48 ± 0.3398). Extractive value of a drug in specific solvent is an index of purity of a drug and plays a major role to determine adulteration.

Ash value is an important parameter for detection of adulteration and impurities. The total ash value and sulfated ash was found to be high which may be attributed to the presence of large amount of silica and other inorganic constituent in two drugs, i.e. Tabasheer and Gile Armani. Moisture content or LOD was found to be 6.027 ± 0.1641. LOD indicates the amount of water and volatile substances present in a particular drug. If any drug has more moisture level, it becomes ideal medium for growth of different types of bacteria and fungi affecting the purity, quality, and efficacy of a drug.

The pH of 1% and 10% solution was found to be 5.450 ± 0.08021 and 25.57 ± 0.486 min and 24.72 ± 0.1881 min. This test represents breakdown of tablets into smaller particles and shows that QT disintegrate within permissible limit (maximum 30 min) when placed in liquid medium in the experimental circumstance.

The mean percentage of the successive extractive values was found maximum in alcohol (10.93 ± 0.32). The mean percentage of the nonsuccessive extractive values was found maximum in water (27.67 ± 0.5783) followed by ethyl alcohol (13.48 ± 0.3398). Extractive value of a drug in specific solvent is an index of purity of a drug and plays a major role to determine adulteration.

| Extract               | Number of peaks at λ max 280 nm | Number of peaks at 254 nm | Number of peaks at 366 nm |
|-----------------------|---------------------------------|---------------------------|---------------------------|
| Petroleum ether       | 7                               | 6                         | 5                         |
| Chloroform            | 13                              | 14                        | 12                        |
| Ethanol               | 10                              | 11                        | 11                        |
| Methanol              | 15                              | 14                        | 5                         |
| Water                 | 12                              | 11                        | 10                        |

Mobile phase - Toluene: ethyl acetate: formic acid (5:4:1)

**Table 4:** High-performance thin layer chromatography peaks for all extract

| Peak number | Area (AU) | Area (%) | Height (AU) | Rf   |
|-------------|-----------|----------|-------------|------|
| 1           | 26,883.4  | 16.87    | 737.0       | 0.07 |
| 2           | 11,331.4  | 7.24     | 399.3       | 0.31 |
| 3           | 8143.5    | 5.11     | 341.0       | 0.17 |
| 4           | 11,785.7  | 7.40     | 327.8       | 0.22 |
| 5           | 16,633.1  | 10.44    | 327.8       | 0.31 |
| 6           | 15,711.4  | 9.86     | 321.3       | 0.36 |
| 7           | 15,826.9  | 9.93     | 402.2       | 0.43 |
| 8           | 21,378.7  | 13.42    | 427.7       | 0.53 |
| 9           | 3350.4    | 2.10     | 97.6        | 0.62 |
| 10          | 3116.8    | 1.96     | 81.8        | 0.68 |
| 11          | 5313.9    | 3.33     | 99.1        | 0.78 |
| 12          | 6688.1    | 4.31     | 148.8       | 0.88 |
| 13          | 6355.0    | 3.99     | 160.3       | 0.93 |
| 14          | 6452.0    | 4.05     | 148.3       | 1.02 |

**Table 5:** Peak list of chloroform extract of Qurse Tabasheer at UV 254 nm

| Peak number | Area (AU) | Area (%) | Height (AU) | Rf   |
|-------------|-----------|----------|-------------|------|
| 1           | 30,865.8  | 15.42    | 766.9       | 0.07 |
| 2           | 24,694.0  | 12.34    | 472.6       | 0.13 |
| 3           | 13,344.7  | 6.67     | 390.4       | 0.22 |
| 4           | 21,294.0  | 10.64    | 399.3       | 0.31 |
| 5           | 20,016.6  | 10.00    | 402.5       | 0.36 |
| 6           | 19,073.1  | 9.53     | 452.4       | 0.43 |
| 7           | 31,854.6  | 15.92    | 599.2       | 0.53 |
| 8           | 3880.2    | 1.94     | 120.2       | 0.62 |
| 9           | 3950.5    | 1.97     | 100.4       | 0.68 |
| 10          | 7607.0    | 3.80     | 129.6       | 0.78 |
| 11          | 13,169.7  | 6.58     | 277.2       | 0.89 |
| 12          | 5560.7    | 2.78     | 164.7       | 0.95 |
| 13          | 4814.1    | 2.41     | 134.3       | 1.02 |

**Table 6:** Peak list of chloroform extract of Qurse Tabasheer at UV 280 nm

**Table 7:** Peak list of chloroform extract of Qurse Tabasheer at UV 366 nm

| Peak number | Area (AU) | Area (%) | Height (AU) | Rf   |
|-------------|-----------|----------|-------------|------|
| 1           | 25,338.1  | 17.81    | 771.0       | 0.07 |
| 2           | 16,502.7  | 11.60    | 524.0       | 0.13 |
| 3           | 11,278.0  | 7.93     | 486.8       | 0.17 |
| 4           | 15,568.6  | 10.94    | 433.2       | 0.21 |
| 5           | 34,559.1  | 24.29    | 382.5       | 0.30 |
| 6           | 15,244.1  | 10.72    | 362.5       | 0.43 |
| 7           | 13,461.8  | 9.46     | 233.9       | 0.53 |
| 8           | 3102.5    | 2.18     | 88.5        | 0.62 |
| 9           | 2161.2    | 1.52     | 61.9        | 0.67 |
| 10          | 2534.4    | 1.78     | 64.7        | 0.76 |
| 11          | 1268.1    | 0.89     | 41.5        | 0.80 |
| 12          | 1243.3    | 0.87     | 27.4        | 0.99 |
diabetes. Hence, the anti-diabetic activity of QT may be attributed to phenols, tannins, and others.

TLC analysis is one of the best methods for characterization and standardization of herbal drugs. The number of spots and Rf value of each spot in a particular mobile phase is an index of identity, purity, and quality of a drug and plays a major role to determine adulteration in drug. Keeping this point in mind, TLC study was done for all the extracts and maximum peaks were seen in Chloroform and Methanol extract [Table 4].

HPTLC fingerprinting is a suitable for rapid and simple authentication and comparison of different herbal formulations. The unique characteristic finger print in terms number of peaks, their Rf values and area under the curve can play a role in monitoring the quality, consistency, and stability of the product. We have reported the HPTLC data for QT for the 1st time in this study. The peak detail of chloroform extract at 254, 280, and 366 nm are reported which will be useful in standardization of the product.

Total fungal and total bacterial count/g was found to be 190 CFU and 2100 CFU, respectively which is under permissible limit. Specific pathogens such as E. coli, Salmonella spp., S. aureus, P. aeruginosa were found to be absent. Thus, it may be concluded that ash values, extractive values and HPTLC analysis at 280, 254, and 366 nm could be used as important parameters for standardization of the formulation. QT has not been standardized using these parameters in any previous work, so present work may act as a reference for its future evaluation.

There are some exceptions and lack of corrections which should be taken care of in future work including work on various pharmaceutical procedures such as further reduction of disintegration time, detailed Assay, test for heavy/toxic metal, pesticide residue (Organochlorine and organophosphorus pesticides, and pyrethroids), test for aflatoxins (B1, B2, G1, and G2). Metallic content of this formulation can vary due to the use of clay that is obtained from various sources like from iron or lead ore etc. Though this formulation is not purely herbal, it contains

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Table 8: Estimation for microbial contamination and specific pathogens

| Parameters                     | Results  | Limits (as per Ayurvedic Pharmacopoeia of India part II) |
|--------------------------------|----------|----------------------------------------------------------|
| Total fungus count/g           | 190 CFU  | 1000 CFU                                                 |
| Total bacterial count/g        | 210 CFU  | 100,000 CFU                                              |
| *Escherichia coli*/10 g        | Absent   | Absent                                                   |
| *Salmonella* spp./10 g         | Absent   | Absent                                                   |
| *Staphylococcus aureus*/10 g   | Absent   | Absent                                                   |
| *Pseudomonas aeruginosa*/10 g   | Absent   | Absent                                                   |
mineral/clay, so it can be mentioned under herbo mineral category. Heavy metal content guidelines are for formulation of herbal and/or animal origin. A new amended guideline is needed by experts for herbo-mineral category of formulation, as they are studied as drugs intended to be used in humans. In the present formulation, preservatives have not been used and moisture level noted by LOD was 6.027 ± 0.1641, this procedure may also include volatile content of the drug. Further development in the formulation need to be spore formation, condition of storage and packaging needs further evaluation with accelerated stability and sophisticated pharmaceutical study.

CONCLUSION

Data for standardization of Qurs Tabasheer were developed and can be used as standards for future evaluation and reference.

Acknowledgment

The authors would like to express their thanks to Prof. M.A Siddiqui, Director, National Institute of Unani Medicine (NIUM) Bengaluru, for providing all the essential assistant and motivation to work, to all the Pharmacy staff of NIUM Pharmacy and staff of Al Ameen college of Pharmacy Bengaluru for help in HPTLC work.

Financial support and sponsorship

This work is a part of Post Graduate research work. Facilities are provided by Director National institute of Unani Medicine, Bengaluru India in terms of drug/chemicals and other infrastructure.

Conflicts of interest

There are no conflicts of interest.

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