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QUALITY ASSESSMENT OF UNANI POLYHERBAL FORMULATION "HABB-E-AZARAQI" BY CONTEMPORARY SCIENTIFIC PARAMETERS

Shazad Yaqub*[1], Mohd. Aftab [1], Mohd. Asif [1] and Peerzada Yasir Yousuf [2]

Department of Ilmul-Advia, Faculty of Medicine (Unani) [1], Jamia Hamdard, New Delhi, India
Department of Botany, Faculty of Science [2], Jamia Hamdard, New Delhi, India

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Correspondence to Author:

Shazad Yaqub

Department of Ilmul-Advia, Faculty of Medicine (Unani) , Jamia Hamdard, New Delhi, India

E-mail: shazadmadhu2011@gmail.com

ABSTRACT: Demand of the Unani herbal drugs is becoming universal as the people are becoming more and more cognisant about the natural benefits of medicines with plant origin. Herbal drugs being easily affordable are becoming the first preference for the common man over purchasing the expensive modern pharmaceuticals. The WHO estimates that 80% of the world population rely on the medicines of plant origin for their primary healthcare. Even though the market for the herbal drugs is increasing with time but the chances of substitution and adulteration have become a major menace to these natural products. In the present study Habb-e-Azaraqi, which is used in the Unani medicine from last several decades for the various ailments of the human body has been evaluated for the various parameters of standardization for the development of its quality standard. The parameters include ash value, moisture content, swelling index, extractive value and quantitative study of various biologically important constituents like phenolic compounds, flavonoids, alkaloids, etc. The study might provide practical information regarding its identification and its pharmaceutical standards.

INTRODUCTION: Habb-e-Azaraqi is one of the imperative poly herbal formulations used in Unani system of medicine from the last several decades. The formulation is cited in various Unani pharmacopoeias like Qarabaddin azam[1], Qarabaddin-e-Majeedi [2], NFUM [3] (National formulary of Unani medicine) etc. In addition, the treatment of infectious diseases is more complicated in immuno-suppressed patients, such as those infected with the HIV, undergoing anticancer therapy and organ transplants.

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The compound drug has been found clinically beneficial for various ailments of human body like falij (paralysis), laqwa (facial palsy), niqrus (gout) [4] and waja-ul-mufasil (arthritis) [5]. All the individual drugs in the compound mixture contain some biologically important chemical constituents like phenolic compounds, flavonoids, tannins, alkaloids, etc. In the present era the lack of proper production, inefficient supply system of crude material and increasing demand are the major factors promoting the practices of adulteration and substitution of such weighty products.

In the present study, the drug was prepared as per the national formulary of Unani medicine and was evaluated for the various parameters of standardization like ash value, moisture content, swelling index, extractive values (Hot and successive extracts).

Quantitative study of various biologically significant constituents like phenolic compounds, flavonoids, alkaloids has been done to support its clinical importance in various diseases. HPTLC analysis was done for methanolic extract of formulation to develop quality standard of this modus operandi.

MATERIALS AND METHODS:

Preparation of Habb-E-Azaraqi: The drug was prepared as mentioned in the NFUM following the WHO guidelines of GMP6.

Physiochemical Studies: Different physiochemical values such as ash values (total ash, acid insoluble ash, and water-soluble ash), loss on drying, extractive value (Hot and successive extract), were determined according to the standard methods [7].

Fluorescence Analysis: Chemical tests of powder drug with different regents were performed to observe the colour reactions according to the reported method [8].

Determination of Total Alkaloid Contents: Accurately weighed (about 10-20 gm) drug was extracted repeatedly with 0.5N H_2SO_4 till the complete extraction of alkaloids was achieved (as tested using Dragendroff's reagent). The acid extract was made alkaline by adding excess of dilute NH_4OH solution. The alkaloids were extracted completely by shaking the alkaline solution with a mixture of chloroform and ether (2:1 ratio) in a separating funnel. The chloroform/ ether extracts are extracted with 20, 15, and 10 ml of 0.5 N H_2SO_4. The combined acid extracts are filtered into a separating funnel and made alkaline with dilute ammonia solution.

The alkaloids in the solution are extracted with successive portions of 20, 15, 10 and 05 ml of chloroform, to ensure their complete extraction. The combined chloroform extract is washed with two 10-ml portions of water. The washings are extracted again with two 10 ml portions of chloroform. The chloroform washing is added to the main chloroform extract and filtered in a tarred 100 ml conical flask. The chloroform is distilled off the chloroform on a water bath till a few ml are left. The solvent is removed completely in a vacuum desiccator.

5ml of alcohol (90%) is added to the residue and the solvent removed. The step is repeated once again. The residue is dried under vacuum till a constant weight is achieved and weighed as total alkaloids [9].

Determination of Total Phenolic Contents: The phenol was determined in powdered crude drugs, extracts and beverages by Folin Ciocalteu method. Standard stock solution was prepared by dissolving 25 mg of catechin standard in 100 ml distilled water. Different concentrations of the standard solutions were prepared for standard calibration curve starting from 4 to 24µg/ml in water. The commercial Folin Ciocalteu reagent was diluted (1: 10 ratio) with distilled water on the day of use. 1M sodium acetate was prepared by dissolving 82 g of sodium acetate in 1000 ml distilled water.

Sample preparation: 500 mg of the samples were taken in 50 ml volumetric flasks. 25 ml of distilled water was then added to them and solicited for 10 minutes and then made up the volume with water.

Procedure: 3ml of each standard and sample solution were taken in 10ml test tube and to it were added 3 ml of FC reagent and 3 ml of sodium carbonate solution. A blank solution was prepared by adding 3 ml each of distilled water, sodium carbonate solution, and FC reagent in a test tube. The solution was kept in dark for 30 minutes for colour development. Absorbance was taken at 415 nm against blank solution. After taking absorbance of standard dilutions, calibration curve was plotted (**Fig. 1**). Phenolic contents in drug were calculated by using standard calibration curve [10].

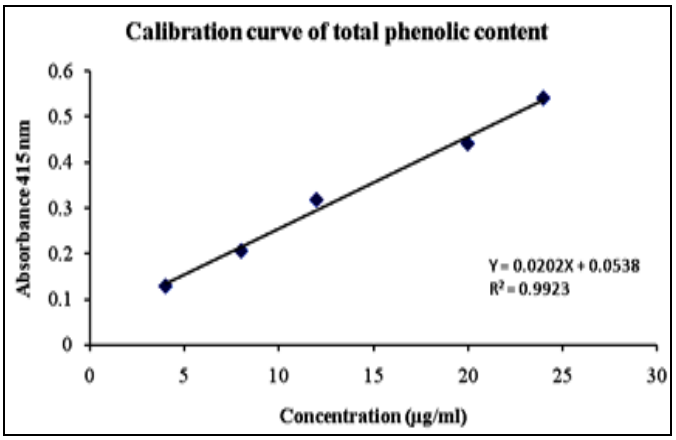


FIGURE 1: CALIBRATION CURVE OF STANDARD CATECHIN FOR TOTAL PHENOLIC CONTENTS

Determination of Total Flavonoid Contents: The flavonoid content was determined in powdered crude drugs according to method described by Pourmorad *et al* [7]. $AlCl_3$ (0.1 g/ml) and CH3COONa (1M) reagents were prepared and then dilutions for Rutin (standard) from 10µg /ml to 100µg/ml were prepared.

Samples Preparation: 500 mg of the samples were taken in 50 ml volumetric flasks and to it was added around 25 ml of methanol water and solicited for 30 minutes and then the volume was made up with methanol.

Procedure: 0.5ml of each standard and sample solution was taken in a 10 ml test tube and 1.5 ml methanol was added to it. 0.1ml of $AlCl_3$ and 0.1 ml of CH_3COONa reagents were then added to it along with 2.8 ml distilled water and kept for 30 minutes. A blank solution was prepared by adding 2 ml of methanol, 0.1ml of $AlCl_3$, 0.1ml of CH_3COONa reagents and 2.8 ml distilled water. Absorbance was taken at 415 nm against blank solution. After taking the absorbance of standard dilutions, calibration curve was plotted (**Fig. 2**). Flavonoid content in drug was calculated by using standard calibration curve [11].

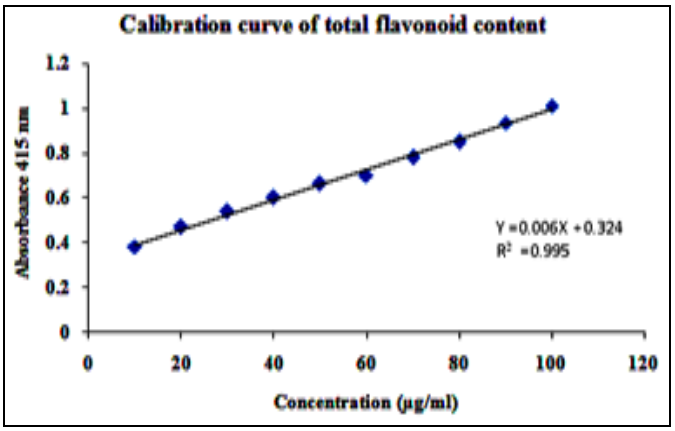


FIGURE 2: CALIBRATION CURVE OF STANDARD RUTIN FOR TOTAL FLAVONOID CONTENT

HPTLC Analysis:

Preparation of Drug Extract for HPTLC Analysis: The dried and coarsely powdered material of Habb-E-Azaraqi (50g) was subjected to successive extractions in a Soxhlet apparatus with different solvents like petroleum ether, chloroform, methanol and water. The residue of methanolic extract obtained was stored in the deep freezer at –200C until further application.

Development and determination of the Solvent system:

- Sample Applied: Sample drug solution of about 5 µl.
- Solvent system: Toluene: Ethyl acetate: formic acid (8:1:1 ratio)
- Migration distance: 80 mm
- Scanning wavelength: UV 366 nm and UV 254 nm The sample of 5 µl each was applied on TLC aluminium sheets silica gel 60 F 254 (Merck) with band length 10nm using sample applicator (Linmat 5), set at the speed of 100nm/Sec. After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated above was selected in its proportionate ratio and developed in the Twin through chamber of TLC to the maximum height of the plate so that it could separate the components on the immiscible polar and mobile phases of silica gel and solvent system respectively. Three batches of formulation were spotted separately and the TLC plate was developed as shown in **Figure 3**.

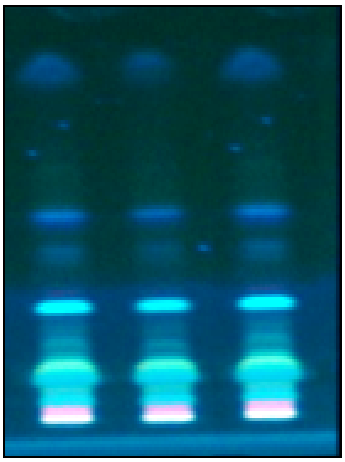


FIGURE 3: TLC OF HABB-E-AZARAQI

Development of the HPTLC Finger Print profile of different extracts: The plant material was coarsely powdered and extracted in Soxhlet apparatus for 6-24 h using solvent petroleum, chloroform and methanol extracts. The extracts were evaporated to dryness in the rota vapour and the solvents were recovered. Gummy residues so obtained, were stored in the deep freezer at –20°C degree until further application.

The samples were prepared by dissolving each extract in their respective solvents to get the concentration of 10 mg/ml. These solutions were further passed through syringe filter to remove any impurity and applied on TLC plate for finger printing analysis.

The extract was applied on HPTLC aluminium sheets silica gel 60 F 254 (Merck) 10 micro l each with the band length 6 mm using Linomat5 sample applicator set a speed of 100nl/sec [12]. Different solvent systems were used for the separation of the constituents of the different extracts. The chromatogram was developed in the twin chamber for 20 minutes up to the distance of 80mm and the spots were visible at 254 and 366nm wavelengths as shown in **fig. 4** and 5 respectively.

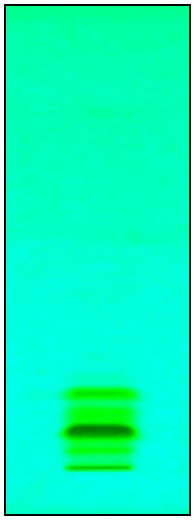


FIG. 4

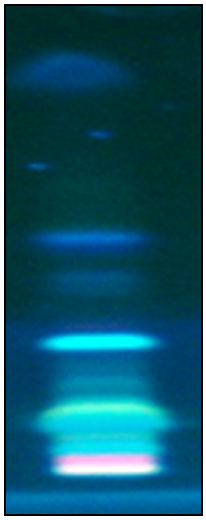
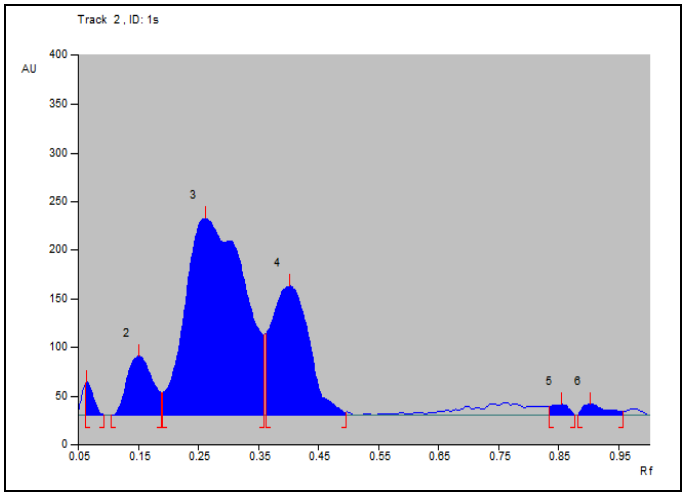
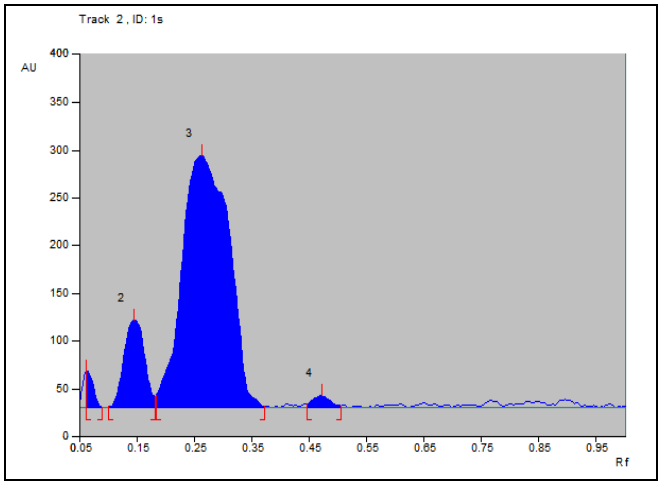


FIG. 5



HPTLC DENSITOGRAM OF SUCCESSIVE METHANOLIC EXTRACT OF HABB-E-AZARAQI AT 254 nm



HPTLC DENSITOGRAM OF SUCCESSIVE METHANOLIC EXTRACT OF HABB-E-AZARAQI AT 366 nm

RESULTS AND DISCUSSION:

Organoleptic Characteristics: The drug is in the form of pills with brownish black colour and has disagreeable smell and bitter taste.

Physicochemical Analysis:

TABLE 1: PRELIMINARY PHYSICOCHEMICAL STUDIES OF THE TEST DRUG

S. No.	Parameters	Values
1	**Ash value**	
*	Total ash	1.20 % w/w
*	Acid insoluble ash	1.00 % w/w
*	Water soluble ash	0.29 % w/w
2	**Loss on drying of crude drug**	7.8 % w/w
3	**Swelling index**	3.52 ml
4	**Extractive values (Hot extract)**	% Extractable matter
*	Petroleum ether extract	8.1 w/w
*	Chloroform extract	2.3 w/w
*	Methanol extract	6.3 w/w
*	Aqueous extract	18.3 w/w
5	**Extractive values (Successive extract)**	% Extractable matter
*	Petroleum ether extract	8.1 w/w
*	Chloroform extract	1.2 w/w
*	Methanol extract	5.3 w/w
*	Aqueous extract	9.2 w/w

Fluorescence Analysis: Chemical tests of the powdered crude drug with different reagents (ethyl acetate, alcohol, Toluene, HCl, HNO_3, Glacial acetic acid, H_2SO_4, I_2) were studied in the day light, UV light (254 and 366 nm). The results obtained are presented in **table 2**.

TABLE 2: POWDERED DRUG REACTION WITH DIFFERENT REAGENTS

S. No.	Solvent system	Ordinary light	UV Light (254nm)	UV Light (366nm)
1.	**Ethyle acetate**	Green	brown	Green
2.	**Alcohol**	Light green	Light brown	Green
3.	**Toluene**	Yellowish green	Brown	Green
4.	**HCl**	Brown	Black	Dark brown
5	**HNO_3**	Orange	Dark purple	Yellow
6.	**Glacial acetic acid**	Yellowish green	Brownish yellow	Yellowish
7.	**H_2SO_4**	Dark purple	Black	Dark purple
8.	**Iodine**	Red	Dark purple	Blackish green

TABLE 3:

Chemical treatment	Observation
Conc. HCl	Dark brown
Conc. HNO_3	Orange
Conc. H_2SO_4	Reddish brown
Ferric chloride	Blackish
NaOH 5%	Blackish brown
Powder as such	Greenish black

Phytochemical Screening of Individual Extracts: The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, tannins, flavonoids. Values obtained are shown in **table 4**.

TABLE 4: TOTAL ALKALOID CONTENT

Chemical	Test	Petroleum	Chloroform	Methanol	Aqueous
Alkaloid	Hager's test	+	_	+	_
	Mayer's test	_	+	+	_
Tannin	Ferric chloride test	-	_	+	+
	Lead acetate test	_	+	+	+
Flavonoids	Shinoda test	+	+	+	+
Glycoside	Borntrager's test	+	+	-	_
Carbohydrates	Fehling's test	+	+	-	-
	Benedict's test	+	-	+	_

The total alkaloid content as determined by the reported method was found to be 1.876% w/w, shown in **table 5**.

Total Flavonoid Content: The results of total flavonoids content of *Nux-vomica* seeds as determined by Aluminium chloride colorimetric method was found to be 0.09 % w/w as is shown in table 5.

Total Phenolic Content: The amount of phenolic content, determined by Folin Ciocalteu method using the standard calibration curve, was found to be 0.38 % w/w as shown in table 5.

TABLE 5: ALKALOID CONTENT, FLAVONOID CONTENT, PHENOLIC CONTENT

S. No.	Chemical Content	VALUES
1	**Alkaloid content**	1.876% w/w
2	**Flavonoid content**	0.09 % w/w.
3	**Phenolic content**	0.38 % w/w

HPTLC Analysis: HPTLC fingerprint studies of methanolic successive extract of Habb-e-Azaraqi for the three batches was carried out and chromatogram with three batches was developed and detected using the UV visible chamber, which clearly showed six spots at UV 254 nm. The corresponding R_f values of the eleven components are at 6 (0.06, 0.15, 0.26, 0.40, 0.85 and 0.90) as

shown in the Tables 4. The R_f values of three batches of the formulation were found to be same and at UV 366 nm four spots were also revealed in densitogram. The R_f values were at 4 (0.06, 0.14, 0.26 and 0.47 as shown in **Tables 6a and 6b**.

TABLE 6A: HPTLC FINGER PRINT OF METHANOLIC EXTRACT OF HABB-E-AZARAQI AT 254 NM

SOLVENT SYSTEM	NO. OF PEAK OBSERVED (R_f)
Toluene: Ethyl acetate: Formic acid (8 : 1 : 1 ratio)	6 (0.06, 0.15, 0.26, 0.40, 0.85, 0.90)

TABLE 6B: HPTLC FINGERPRINT OF METHANOLIC EXTRACT OF HABB-E-AZARAQI AT 366 NM

SOLVENT SYSTEM	NO. OF PEAK OBSERVED (R_F)
Toluene: Ethyl acetate: Formic acid (8 : 1 : 1 ratio)	4 (0.06, 0.14, 0.26, 0.47)

CONCLUSION: Habb-e-Azaraqi is a well-known Unani formulation and is used in this system of medicine from several decades for various clinical pathologies. The compound is mixture of various botanical herbs in specific proportional, which in turn is far more important for its reported clinical efficacy and simultaneously increased demand of this compound. These peculiarities make it susceptible for the adulteration at manufacture level.

Keeping all these problems in consideration, in this study the drug has been prepared according to the GMP guidelines of the WHO. Various standardization parameters like ash value, moisture content, fluorescence analysis and other physical parameters along with the HPTLC have also been carried out so that scientific data for its originality can been provided for further studies of this drug in future.

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