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STANDARD OPERATING PROCEDURES (SOPs) FOR THE DEVELOPMENT OF UNANI POLYHERBAL FORMULATION "HABB-E-AZARAQI" AND ITS PHYSICOCHEMICAL ANANLYSIS

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ABSTRACT

Multi-herbs dominate as the largest segment, capturing a significant share of the overall herbal supplements and remedies market worldwide. The global herbal supplements and remedies market has been forecasted to reach about US$ 93.15 billion by the year 2015, spurred by increasing incidence of aging population and consumer awareness about general health and well being. Herbal product has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of these formulations is the lack of standard operating procedures (SOPs) for their development and quality control profile. Keeping the present demand of herbal products in view, in the present study, the poly herbal formulation Habb-e-Azaraqi which is commonly used in paralysis and arthritis has been developed by SOPs and various standardizing parameters has been evaluated, so as to ascertain its quality.

INTRODUCTION

Multi herbal formulations dominate as the largest segment, capturing a significant share of the overall herbal supplements and remedies market worldwide. The global herbal supplements and remedies market has been forecasted to reach about US$ 93.15 billion by the year 2015. In present advanced era, an increased demand of poly herbal formulations suggests a great need of standard criteria for the development and quality control of these herbal preparations. WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and by applying suitable parameters and standards. In order to overcome certain inevitable shortcomings of the pharmacopoeial monograph, other quality control measures must also be explored[1].

A poly herbal formulation, Habb-e-Azaraqi used in present study is a Unani product mentioned in National formulary of Unani medicine (NFUM)[2].The drug is used in the treatment of various ailments of human body, especially the nervous disorders like paralysis and is also used as an anti arthritic medicine from a long time[3].

In order to standardize the drug and to lay out the SOPs for its preparation, the formulation has been prepared in three batches at the lab scale by adopting the GMP guide lines of WHO. The drug has been evaluated for various parameters like morphological and microscopic studies, physicochemical parameters like ash value, moisture content, pH of 1% and 10% solutions, disintegration time, pill friability, aflotoxin, heavy metal analysis, microbial load, HPTLC analysis[4]. The present study provides an essential reference model for the preparation of the formulation and also describes salient features of identification and safety evaluation of the product.

METHODS: Preparation of Habb-e-Azaraqi by SOP:

Habb-e-Azaraqi formulation has been prepared by standard operating procedures (SOPs) as per the WHO guidelines for good manufacturing procedure (GMP)[5]. The SOP involves the following steps:

1. Foreign matter analysis of crude drugs:

All the individual crude drugs Azaraqi (*Strychnos nux-vomica* linn.*)*, Filfil siyah *(Piper nigrum* linn.*)*, Filfil daraz *(Piper longum* linn.*)*, and Ajwain *(Trychespermum ammi* linn*)*, were procured

from the Khari bawli, old Delhi 110006. Each drug was separately weighed to 250g and was spread on a thin layer on white paper. All the foreign matter was inspected with the unaided eye and total weight and percentage of foreign matter was noted for each drug.

2. Authentication of crude drugs:

All the purified drugs samples were separately send in aseptic air tight plastic bottles for identification. The identity of all the drugs was established by National institute of science communication and information resources (NISCAIR),CSIR, Dr K.S .Krishnan Marg, near Pusa Gate, New dehli-110012. Voucher specimen Ref. No. of the voucher is NISCAIR/RHMP/F-3/Conlt/o6/720/37, dated 14/12/2009 which was retained and deposited for the future references in the department of Ilmul Advia, Faculty of medicine (Unani), Hamdard University, New Dehli-110062.

4. Detoxification of Azaraqi:

The purified seeds of Azaraqi 500 gms were soaked in distilled water for seven days. The water was changed daily to avoid fermentation. After seven days of water treatment, the seed coat (testa) was removed and the cotyledons (endosperm) were separated, after removing the embryo part. 60 gms of water treated Azaraqi seeds (cotyledons) were placed in a fine bag. The bag was tied with a thread in fine cloth bag and suspended in 1 kilogram of fresh cow- milk contained in a beaker. The bag was kept in such a position so that its contents remain always immersed in milk and bag should not touch the base of the container. The milk was gradually heated at its boiling point on the hot plate till the whole milk condensed into the khoya (cream). Care was taken so as to lower down the bag time to time so that it would always remain inside the boiling milk. The Azaraqi was then removed from the bag and washed with distilled water. The moist kuchla was cut into the small pieces and placed in the oven at 105^{o}c for two hours to dry [6].

6. Powdering of drugs:

The entire individual drugs were taken and each of the drugs was powdered one by one in the mixer grinder. Before powdering each sample the grinder was properly washed and dried. Then, the powdered drugs were separately sieved with mesh (number 100).

7. Formation of lubdi (dough):

Powder of *Strychnos nux-vomica*, *Piper nigrum*, *Piper longum* and *Trachyspermum ammi* was taken in the 2:1:1:1 ratio respectively by weight and put into the properly cleaned glass container. Then sufficient quantity of water (obtained from distillation plant) mixed with 10% of gum acacia (10% of total weight of all individual drugs) was added to the bulk of the powder and whole powder bulk was mixed thoroughly with the gloved hands to make lubdi.[2]

8. Pill formation:

Lubdi was rolled in the form of sticks of required size and then small pieces from stick were taken and were rounded between the fingers. Each pill was weighed up to 150 gm.

9. Drying of pills:

All the prepared pills were put into the vacuum oven at 105^0 temperature for two hours for drying.

Chemical analysis

Physicochemical parameters such as total ash, moisture content, pH of 1% and 10% solution, disintegration time, pill friability and HPTLC analysis of the prepared compound formulation Habb-e-Azaraqi were studied. The heavy metal, aflatoxin and microbacterial load was analysed as per AOAC 2005, ASTA 1997 and WHO 1998 respectively.

Preparation of extract of the drug for HPTLC analysis

The dried and coarsely powdered material of Habb-e-Azaraqi (50g) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform, methanol and water. The Gummy residue of chloroform extract obtained was stored in the deep freezer at – 20^0C 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)

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 Linmat 5 sample applicator, set at the speed of 100nm/Sec CAMAG, Switzerland). 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 developed the TLC plate as shown in Figure 1.

Figure-1

Figure-1: TLC chromatogram of the chloroform extract of three batches of Habb-e-Azaraqi at UV 366nm observed in UV chamber showing better separation of components (spots)

Development of HPTLC technique

HPTLC was carried out according to the method described by Ergon,1969. The extract (3gm) was dissolved in the silica gel 60 F_{254} (Merck,20mm). Chloroform extractives were subjected to HPTLC analysis in this manner. Activation of HPTLC plates was done in an oven. The samples were applied on the plates in a quantity of 5µl in the form of bands of 10 mm width with the help of camag linomat –V sample applicator by using 100 µl syringe and dried under a nitrogen flow. The chamber was saturated with mobile phase at room temperature for 15 minutes. The plates were developed to a distance of 80 mm in a camag twin trough chamber .The plates were dried and scanned at 254 nm and 366 nm using camag HPTLC scanner 3. The dimension was 0.8 -0.9mm[7].

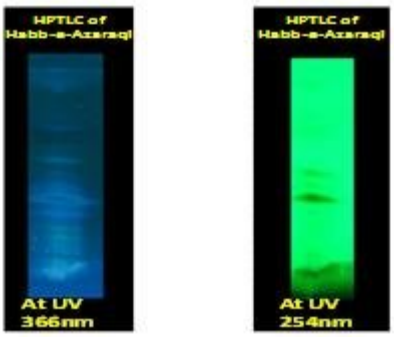

Figure-2: TLC chromatograms of chloroform extract of Habb-e-Azaraqi at different wavelength.

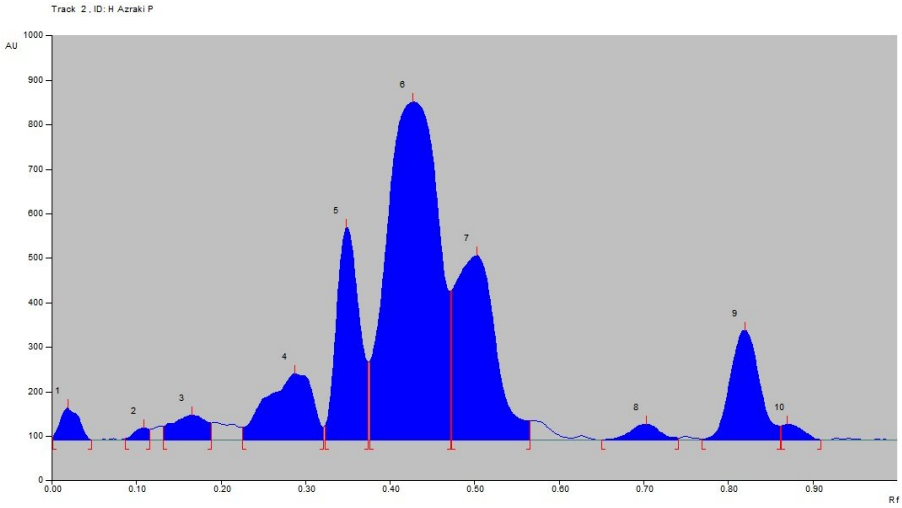

Figure-3: HPTLC Densitogram of the chloroform extract of Habb-e-Azaraqi at 366nm

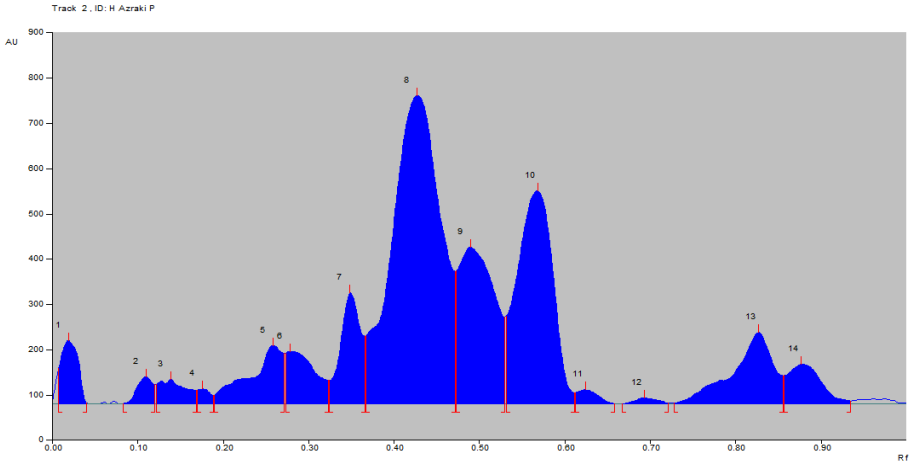

Figure-4: HPTLC Densitogram of the chloroform extract of Habb-e-Azaraqi at 254nm

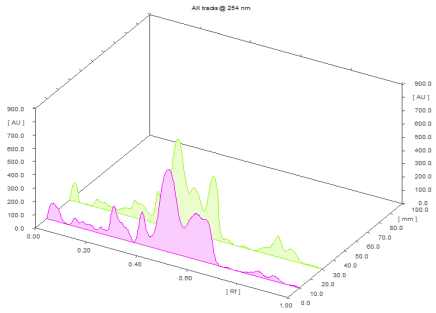

Figure-5: 3D at 254nm

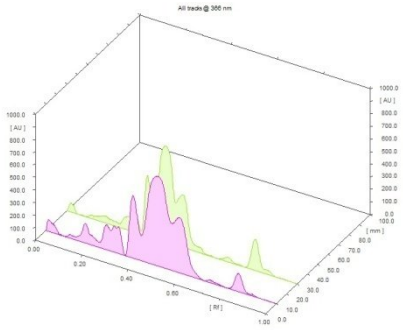

Figure-6: 3D at 366 bnm

RESULTS AND DISCUSSION

Organoleptic characteristics:

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

Microscopic powder study:

The preparation under high power of microscope shows fragmented lignified rods of trichomes, abundant endosperm cells, large number of aleurone grains and scattered Schlarenchymatus cells (Azaraqi), elongated parenchymatus cells filled with starch grains, isodiametric, elongated and scattered stone cells (Filfil Daraz), lot of parenchymatus cells of different shape and size, with small vessels, trachieds and fibres (Ajwain), numerous isodiametric, coloumnar, horse shoe shaped stone cells (Filfil siyah).

Physicochemical Standards

Physicochemical Parameters data shown in **Table 1** is expressed as mean values of the five readings calculated. **Table 1** Physico-chemical parameters of the compound formulation 'Habb-e-Azaraqi'

	Parameters	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Mean value
1.	**Ash value**						
a	**Total ash**	6%	5.8%	5.8%	5.7%	6.1%	**5.90%**
b	**Water soluble ash**	1.2%	1.24%	1.25%	1.30%	1.15%	**1.22%**
C	**Acid insoluble ash**	2.82%	2.72%	2.08%	2.62%	2.56%	**2.56%**
2	**Moisture content**	8%	7%	8.4%	9.4%	7%	**7.96%**
3.	**Ph 1%**	6.99	6.87	6.81	6.8	7	**6.89**
4.	**Ph 10%**	6.87	6.29	6.12	6.58	6.44	**6.46**
5.	**Disintegration time**	17 sec	22 sec	19 sec	17 sec	23 sec	**19.6 sec**
6.	**Friability test**	14.36%	13.40%	16.16%	16.56%	13.20%	**14.73%**

Analysis of the microbial load: Table 2 Microbial Contamination

1	Total bacterial count(TBC)	4×10^3 CFU/g	10^5 CFU/g
2	Total fungal count(TFC)	3×10^2 CFU/g	10^3 CFU/g
3	*Enterobacteriaceae*	Absent	10^3 CFU/g
4	*Escherichia coli*	Absent	10 CFU/g
5	*Salmonella Spp*	Absent	Absent
6	*Styphylococcus aureus*	Absent	Absent

Table 3 Aflatoxin Contamination

Aflatoxin analysis:

S.NO	Aflatoxin	Results
1	B1	Not detected
2	B2	Not detected
3	G1	Not detected
4	G2	Not detected

Table 4 Heavy Metal Analysis

Heavy metal analysis:

S.NO	Name of element	Results	Permissible limit
1	Lead	0..220ppm	10ppm
2	Cadmium	0.0288ppm	0.3ppm
3	Mercury	0.0021ppm	1ppm
4	Arsenic	0.0002ppm	3ppm

HPTLC analysis

HPTLC fingerprint studies of chloroform successive extract of Habb-e-Azarqi for the three batches was carried out and chromatogram with three batches was developed and detected using the UV visible chamber, which clearly showed eleven spots at UV 366 nm. The corresponding R*f* values of the eleven components are at, 0.02, 0.11, 0.17, 0.29, 0.35, 0.43, 0.59, 0.70, 0.82 and 0.87 as shown in the Tables 4. The Rf values of three batches of the formulation were found

to be same and at UV 254nm fourteen spots were also revealed in densitogram. The R*f* values were at 0.02, 0.11, 0.14, 0.18, 0.26, 0.28, 0.35, 0.43, 0.49, 0.57, 0.62, 0.69, 0.83 and 0.88 as shown in Tables 5.

Table: HPTLC fingerprint of chloroform extract of Habb-e-Azaraqi at 366 nm

Solvent system	No. of peak observed (R_f values)
Toluene : Ethyl acetate : Formic acid (8 : 1 : 1)	10 (0.02, 0.11, 0.17, 0.29, 0.35, 0.43, 0.59, 0.70, 0.82, 0.87)

Table: HPTLC fingerprint of chloroform extract of Habb-e-Azaraqi at 254 nm.

Solvent system	No. of peak observed (R_f)
Toluene : Ethyl acetate : Formic acid (8 : 1 : 1)	14 (0.02, 0.11, 0.14, 0.18, 0.26, 0.28, 0.35, 0.43, 0.49, 0.57, 0.62, 0.69, 0.83, 0.88)

CONCLUSION

In the present study the poly herbal formulation has been prepared according to GMP guide lines of WHO at the small scale which provides a reference model for preparation of this product. The drug under study was also subjected to physicochemical analysis, which is helpful in establishing the standard along with the other parameters like microscopic study etc. Heavy metal analysis and aflatoxin contamination were done and were found absent as reported in the present investigation. Besides, the presence of microbial load was found within the permissible limits of WHO guidelines. Modern technique of HPTLC analysis was employed in respect to standardization and to separate the compounds which can be isolated for further studies. The study is likely to help in the quality assurance of drug used in the Unani System of Medicine and in the development of standard parameters.

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