Standardization and HPTLC Fingerprinting of a Unani Compound Formulation 'Qurs-e-Luboob' with Modern Techniques

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Abstract

With global realization that use of synthetic drugs is not safe on the long run, the medical fraternity at large is looking at alternatives from natural sources to combat diseases particularly those in which conventional modern system of medicine has little to offer. This realization on the one hand has increased demand for herbal drugs and on the other hand need for quality standardization of drugs has gone up. Central Research Institute of Unani Medicine, Hyderabad being engaged in multidisciplinary research in Unani Medicine, working on standardization of herbal drugs used in this system of medicine. One such drug "Qurs-e-Luboob" which is prescribed in Unani system for therapeutic use as Zof-e-Bah (Sexual Debility), Qillat-e-Mani (Oligospermia), and has action as Muqawwi-e-Bah (Aphrodisiac), Mughalliz-e-Mani (Inspissant to Semen), has been taken up for standardization by modern techniques, so as to ascertain its quality. The parameters carried out are pharmacognostic studies, physico-chemical parameters, phytochemical screening, High performance thin layer chromatography, microbial load, aflatoxins, and heavy metals revealing specific identities for the particular drug and to evaluate pharmacopoeial standards. Results suggest that the drug is safe for therapeutic use and its batch to batch identification for quality control is possible on the basis of present study.

Keywords: Qurs-e-Luboob, Standardization, Physico-chemical analysis, HPTLC.

Introduction

Recently, there has been a shift in universal trend from synthetic to herbal medicine, which can be said 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments (Sharma *et al*., 2008). The global resurgence of interest in herbal medicines has led to an increase in their demand leading to a decline in their quality, primarily due to a lack of adequate regulations pertaining to drugs (Rajini and Kanaki, 2008). WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques (Imam *et al*., 2009; Rasheed *et al*., 2010a; 2010b; 2010c;2010d; 2011; 2012; 2014a; 2014b) and (Naikodi *et al*., 2011) by applying suitable parameters and standards, In order to overcome certain inevitable shortcomings of the pharmacopoeial monographs other quality control measures must be explored (Shinde, 2009; Singh and Soni, 2004; Street *et al*., 2008). Curative efficacies of compound herbal medicine are reliant on the quality and

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the quantity of the constituent single drugs as they contain specific bio-active marker species with specific pharmacological actions. Though, it is very difficult to identify the ingredients after the formulation is prepared and the organoleptic parameters like taste, odour, colour etc. will not establish the standard quality of the medicine.

A Unani formulation, Qurs-e-Luboob (figure 1) was taken for the present study. The drug is a compound formulation mentioned in National formulary of Unani medicine of India, Part-IV. and prescribed for the treatment of Zof-e-Bah (Sexual debility), Qillat-e-Mani (Oligospermia), and Muqawwi-e-Bah (Aphrodisiac), Mughalliz-e-Mani (Inspissant to Semen).

In order to standardize and to lay down the standard operating procedures (SOP's) and pharmacopoeial standards, the formulation was prepared in three batches at laboratory scale. It was subjected to analysis for microscopic study, physico-chemical parameters, microbial load, heavy metals, aflatoxins, and high performance thin layer chromatographic studies (Anonymous, 2009). The present paper describes the salient features of preparation, phytochemical screening, safety evaluation studies and High performance thin layer chromatographic studies for the drug.

Materials and Methods

Collection of material

Ingredients of formulation were procured from the pharmacy of Central Research Institute of Unani Medicine, Hyderabad, authenticated and identified by the expert botanist.

Fig. 1: Formulation Qurs-e-Luboob (*tablets*).

Preparation of the formulation as per Sharif Khan (1921 A.D.).

It is prepared according to the composition of the formulation given in national formulary which is as follows:

S.No.	Name of the drug	Botanical Name	Part Used	Qty
1.	Maghz-e-Funduq	*Corylus avellana* Linn.	Cotyledons	100g
2.	Maghz-e-Pista	*Pistacia vera* Linn.	Cotyledons	100g
3.	Maghz-e-Badam shireen	*Prunus amygdalus* Batsch.	Cotyledons	100g
4.	Maghz-e-Behidana	*Cydonia oblonga* Mill.	Cotyledons	100g
5.	Maghz-e-Tukhm-e-Kharbuza	*Cucumis melo* Linn.	Cotyledons	100g
6.	Maghz-e-Tukhm-e-Khiyarain	*Cucumis sativus* Linn.	Cotyledons	100g
7.	Maghz-e-Tukhm-e-Kadu	*Cucurbita moschata* Duchesne.	Cotyledons	100g
8.	Maghz-e-Tukhm-e-Hindu dana	*Citrullus vulgaris* Schrad.	Cotyledons	100g
9.	Tukhm-e-Khashkhash safaid	*Papaver somniferum* Linn.	Seed	100g
10.	Maghz-e-Habb-e-Mahlab	*Prunus mahaleb* Linn.	Cotyledons	100g
11.	Nishasta	*Triticum aestivum* Linn	Starch	100g
12.	Tukhm-e-Khatmi	*Althaea officinalis* Linn.	Seed	100g
13.	Tukhm-e-Khubbazi	*Malva sylvestris* Linn	Seed	100g
14.	Rubbus-soos	*Glycyrrhiza glabra* Linn.	Root extract	100g
15.	Gil-e-Armani	Armenian bole	Aluminium Silicate, Silicate of Alumina, Magnesia andIron Oxide	100g
16.	Maghz-e-Chilgoza	*Pinus gerardiana* Wall.	Cotyledons	100g
17.	Duqu	*Peucedanum grand* C.B. Clark	Seed	100g
18.	Badiyan	*Foeniculum vulgare* Mill.	Fruit	100g
19.	Tukm-e-Karafs	*Apium graveolens* Linn.	Seed	100g
20.	Luab-e-Tukhm-e-Katan	*Linum usitatissimum* Linn.	Seed Mucilage	Q.S.

Processing of raw material

It is prepared according to the composition of the formulation given above is as follows;

Take all the ingredients of pharmacopoeial quality. Clean all the ingredients from 1-10 and 16 and make them free from all foreign matters and make fine powder separately. This forms Part A with the help of pulveriser and pass through 80 mesh sieve. Similarly ingredients from 11-15, 17, 18 and 19 are powdered separately with the help of pulveriser and pass through 80 mesh sieve. This form Part B. Soak Tukm-e-katan in water to make Luab and add part A and part B in it to make granules and prepare the Qurs through mechanical process. Store the Qurs in a sealed container protected from light and moisture.

Preparation of the Qurs (Tablets)

The tablets were prepared as per the procedure described by Hm. Mohd. Sharif Khan, (1921). The granules were made into 500mg tablets (excluding binding material weight) using rotary tablet punching machine (Cadmach-GMP model).

Chemical analysis

Physico-Chemical parameters of the prepared compound formulation Qurs-e-Luboob were studied such as total ash, acid insoluble ash, water soluble ash, solubility matter in alcohol and water, loss on drying at 105^0 C, microbial load, aflatoxins, pesticide residue and GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used to determine the concentration of heavy metals as per the methods described in WHO guidelines (Anonymous, 1998). Phytochemical screening was carried out in different solvents extracts such as Petroleum ether, Chloroform, Ethyl acetate, Ethanol, Acetone and Aqueous extracts as per the methods described by Trease and Evans, (1972).

HPTLC analysis

DESAGA Sarstedt Gruppe system is used for analysis along with Automatic TLC applicator and UV visible cabinet as imaging system, the instrument had Proquant 1.6 version as software system for documentation.

Preparation of extract of the drug for HPTLC analysis

Five grams fine powder of Qurs-e-Luboob was reflux on water bath for 30 min in Petroleum ether ($60\text{-}80^0$C) through sohxlet apparatus. Later the contents were removed and filtered through Whattmann No. 41 filter paper and evaporated the solution to 20 ml. Thus the solution so obtained was used as sample for the determination of components.

Development and determination of the solvent system

Sample Applied	:	Sample drug solution of about 10µl.
Solvent system	:	Toluene: Ethyl Acetate (9: 1)

The sample was spotted with the help of Automatic TLC applicator system of the DESAGA Sarstedt Gruppe on Precoated Aluminium Sheets of Silica Gel 60 F_{254} (Merck) After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated above is selected in its proportional ratio and developed in the Twin through chamber of TLC to the maximum height of the plate so that it can be able to separate the components on the polar phase of silica gel and that of mobile phase of solvent system. The drug extract was spotted on TLC plate and developed the TLC plate.

Development of HPTLC technique

After developing, TLC plates were dried completely and detected with the suitable detection system like UV Cabinet system for detection of spots at 254, 366nm and also under iodine vapours and after derivatizing with anisaldehyde sulphuric acid reagent as shown in the figure 2. Further it was scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 366nm, under exposure to Iodine vapours at 410nm and after derivatization with anisaldehyde sulphuric acid reagent at 580nm showing corresponding densitogram in the figure 3 in which peaks appeared for the corresponding spots being detected in the densitometer while scanning and the peaks areas under the curve correspond to the concentration of the component in the sample. The separation of the components in the compound formulation and its ingredients are corresponding compared with respect to Rf values.

Results and Discussion

Analytical Profile

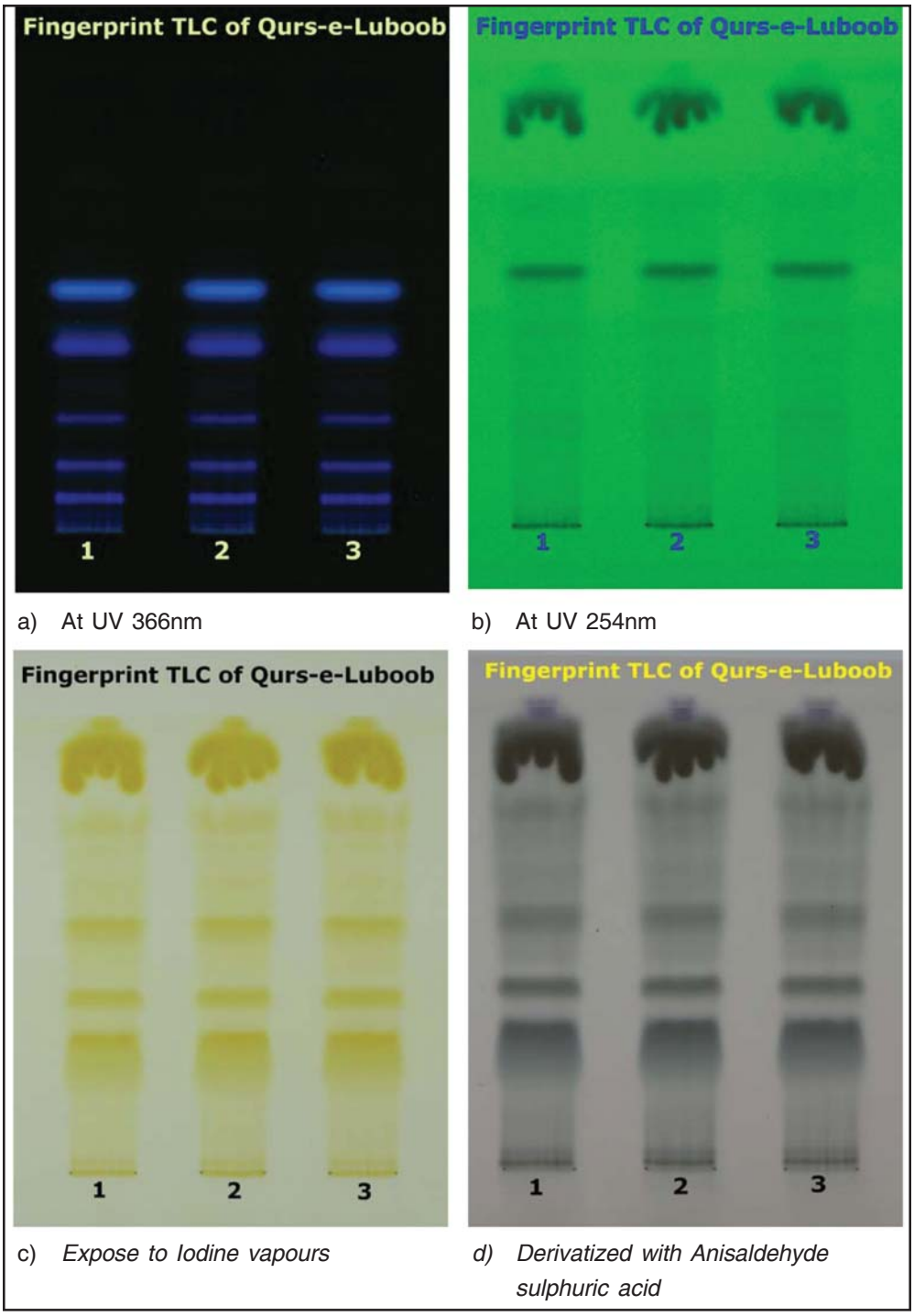
Organoleptic Characters

Light brown colour tablet having characteristic smell.

Identification

Microscopy

Fine powder of six tablets was immersed in the water and kept for half an hour. Material was stirred with a glass rod and supernatant was discarded. Residue



a) At UV 366nm

b) At UV 254nm

c) *Expose to Iodine vapours*

d) Derivatized with Anisaldehyde sulphuric acid

Fig. 2: TLC plates of alcoholic extract of Qurs-e-Luboob a) At UV 366nm, b) At UV 254nm, c) Under Iodine vapours d) At visible region after derivatizing with Anisaldehyde sulphuric acid reagent.

was taken in a glass jar and it was treated with chloral hydrate solution for an hour. After that residue was treated with iodine solution, safranin and mounted with glycerine. Fatherly slide was subjected for microscopical studies.

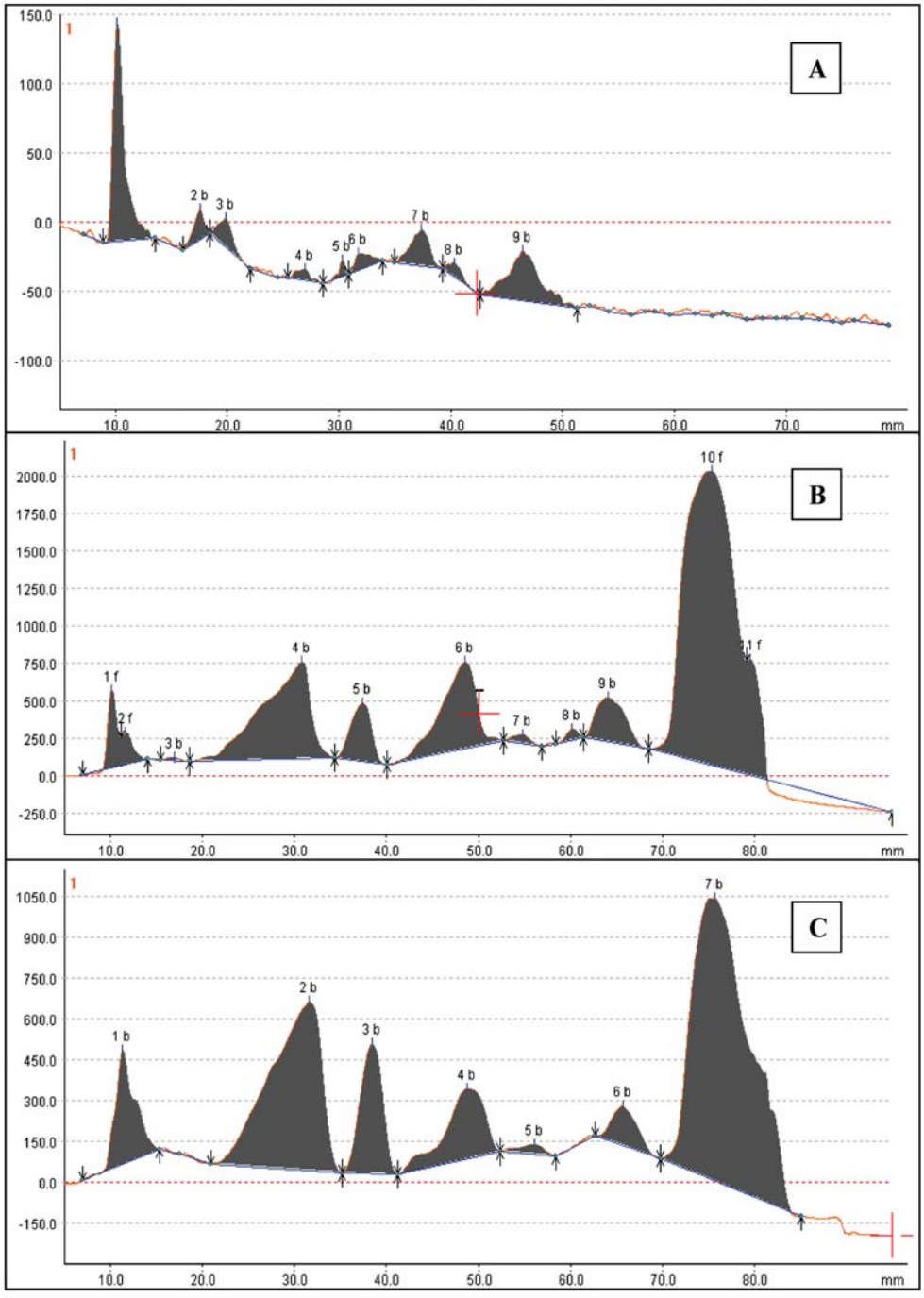


Fig. 3: HPTLC Densitogram of Qurs-e-Luboob at A) UV 366nm B) Under Iodine vapours at 410nm C) derivatized with anisaldehyde sulphuric acid at 580nm.

Qurs-e-Luboob

Microscopical observations expose the presence of unicellular pointed trichomes, thin walled parenchyma cells, spherical shaped parenchyma cells with crystals and starch grains.

Parenchyma cells with reddish tannin content

Physico-Chemical Standards

The Physico-Chemical Parameters data as given in table 1 is expressed as mean values of the three readings calculated. Total ash was found to be 18.45-18.89, and acid insoluble ash 6.25-6.65 gm%; whereas Alcohol soluble matter in terms of %w/w is found to be 16.33- 17.52 and water soluble matter as 32.91-37.52; The moisture content i.e., Loss of weight on drying at 105^0C found to be 3.48-3.53 gm%. P^H of the 1% aqueous solution observed as 6.68-6.79 and 10% aqueous solution observed as 5.29-5.35; and Disintegration time of tablet was 35min. Phytochemical screening for the phytoconstituents were carried out and

Table 1: Physico-chemical parameters of the compound formulation 'Qurs-e-Luboob'.

S.No.	Parameters	Sample I	Sample II	Sample III
1	Ash values Total Ash (%)	18.60-18.75	18.45-18.50	18.75-18.89
	Acid insoluble ash (%)	6.35-6.50	6.25-6.32	6.60-6.65
2	Alcohol Soluble matter (%w/w)	16.80-16.89	16.33-16.44	17.21-17.52
3	Water soluble matter(%w/w)	33.14-36.95	33.54-37.52	32.91-33.51
4	pH of 1% aq. Solution	6.79	6.68	6.70
	pH of 10% aq. Solution	5.29	5.35	5.33
5	Disintegration time in min.	35	34	35
6	Loss of wt. on drying at 1050C.	3.48	3.52	3.53

Table 2: TLC profile of alcoholic extract of Qurs-e-Luboob along with R_f values and detection system.

S. No.	Name of the extract	Solvent system	Detection	No.of spots	R_f values
1.	Alcoholic extract	Tol: Ethyl acetate=9:1	UV 366nm	six spots	0.60 (Blue), 0.16 (Blue), 0.24 (Fluorescent Blue), 0.40 (Red), 0.44 (light blue) and 0.53 (Fluorescent Blue)
			UV 254nm	two spots	0.58, 0.90 (All black)
			Under exposure to Iodine vapours	five spots	0.29, 0.37, 0.53, 0.76, 0.91 (All brown)
			After derivatizing with anisaldehyde sulphuric acid	five spots	0.29, 0.37, 0.53, 0.76, 0.91 (All Grey)

are represented in the table 3. Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight i.e, visible region and under ultra-violet light were noticed and are presented in the table 4. Fluorescence analysis of powdered drug extracts in different solvents was observed and reported in the table 5. The results

Table 3: Phytochemical screening of the nature of compounds present in different solvent extracts of Qurs-e-Luboob.

S. No.	Phyto constituent	Pet. ether ext.	$CHCl_3$ ext.	E.A. ext.	ethanol ext.	Acetone ext.	Aqueous ext.
1.	Alkaloid	-	-	-	-	-	-
2.	Carbohydrates	-	+	-	+	+	+
3.	Glycosides	+	+	+	+	+	+
4.	Phenols	-	-	-	+	+	+
5.	Saponins	-	-	-	-	-	-
6.	Proteins	-	-	-	+	+	-
7.	Starch	-	-	-	-	-	+
8.	Steroids	-	+	+	+	+	-
9.	Flavonoids	-	-	-	-	-	-
10.	Tannins	-	-	-	+	+	+

Table 4: Fluorescence analysis of powdered drug

S. No.	Reagents	Visible light	UV light	
			Short 254nm	Long 366nm
1.	Powder as such	Brown	Black	Pale yellow
2.	Powder treated with 1N HCl	Brown	Black	Light blue
3.	Powder treated with 50% H_2SO_4 aqueous	Dark Brown	Black	Blue
4.	Powder treated with 50% HNO_3 aqueous	Brown	Black	L. Blue
5.	Powder treated with Glacial Acetic acid	Brown	Black	L. Blue
6.	Powder treated with 1N NaOH in Water	Brown	Black	Pale yellow
7.	Powder treated with 1N NaOH in Methanol	Yellow	Black	Indigo

of total bacterial load and total fungal count of the microbial studies were within the permissible limits and the other parameters were found to be absent in the drug. The analysis of aflatoxins and heavy metal analysis showed that the drug was free from any contaminations. These findings as observed for microbial load, aflatoxin contamination and heavy metal analysis are given in table 6 respectively.

Table 5: Fluorescence analysis of powdered drug extracts in different solvents

S.No.	Extraction Solvent	Visible light	UV light	
			Short 254nm	Long 366nm
1.	Petroleum ether extract	Light yellow	Black	Light pink
2.	Chloroform Extract	Light brown	Black	Blue
3.	Ethyl Acetate	Yellow	Black	Blue
4.	Alcoholic Extract	Yellow	Greenish Black	Light blue
5.	Acetone Extract	Yellow	Black	Blue
6.	Distilled water	Brown	Black	Light blue

Table 6: Heavy Metal Analysis, Aflatoxins, Microbial and fungal contamination in the drug.

Heavy Metal Analysis			
	Parameter analyzed	**Results**	**Permissible limits as per WHO**
1	Arsenic	Nil	Not more than 3.0 ppm
2	Cadmium	Nil	Not more than 0.3 ppm
3	Lead	Nil	Not more than 10.0 ppm
4	Mercury	Nil	Not more than 1.0 ppm
Aflatoxin Contamination			
	Parameter analyzed	**Results**	**Permissible limits as per WHO**
1	B1	Nil	Not more than 0.50 ppm
2	B2	Nil	Not more than 0.10 ppm
3	G1	Nil	Not more than 0.50 ppm
4	G2	Nil	Not more than 0.10 ppm
Microbial and fungal Contamination			
	Parameter analyzed	**Results**	**Permissible limits as per WHO**
1	Total *Bacterial* Load	10 x 10^2	Not more than 10^5 / g
2	*Salmonella Spp*	Nil	Nil
3	*Escherichia Coli*	Nil	Nil
4	Total *Fungal* count	4 x 10	Not more than 10^3 / g

HPTLC analysis

HPTLC fingerprint studies of alcoholic extract of Qurs-e-Luboob was carried out and TLC plate developed and detected using the UV visible chamber which clearly showed various spots at UV 254nm and 366nm in the densitogram and also under iodine vapours and after derivatizing with anisaldehyde sulphuric acid reagent. The corresponding Rf values under each detection is illustrated in the table 2. Which showed shows six spots under 366nm at Rf values 0.06 (Blue), 0.16 (Blue), 0.24 (Fluorescent Blue), 0.40 (Red), 0.44(light blue) and 0.53(Fluorescent Blue); and under UV 254nm shows two spots at Rf values 0.58, 0.90 (All black); and upon exposure to Iodine vapours shows five spots at Rf values 0.29, 0.37, 0.53, 0.76, 0.91 (All brown) and under visible region after derivatizing with anisaldehyde sulphuric acid shows five spots at Rf values 0.29, 0.37, 0.53, 0.76, 0.91 (All Grey). Thus established HPTLC fingerprinting profile helps to authenticate the formulation in batch to batch consistency and quality control analysis of formulation as a reference.

Conclusion

The drug under study was subjected to Physico-chemical analysis, which is helpful in establishing the standard along with the other parameters such as phytochemical screening, microscopic study, HPTLC analysis. Safety evaluation of drug such as Heavy metal analysis, aflatoxins contamination analysis was done and found absent; 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 to generate marker for the formulation. Consequently the drug was brought up in determining and ascertaining its quality standard**.** The study is likely to help in the quality assurance of drug used in the Unani System of Medicine and in development of standard parameters. The development of this traditional system of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural herbal products in the healthcare.

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