PHYSICOCHEMICAL AND HPTLC STUDIES OF QURS-E-MULAIYIN – A POTENT UNANI LAXATIVE

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ABSTRACT

The phenomenal growth in the use of herbal medicines has arisen new challenges for ensuring their safety and efficacy. In the manufacturing of poly-herbal formulations, adulteration, contamination or substitution of costly ingredients are familiar practices that adversely affect the quality of the formulations. Hence, a well-defined methodology is essential to evaluate the quality of herbal medicines in order to maintain their reproducible therapeutic effects. The Unani poly-herbal formulation Qurs-e-Mulaiyin, widely used as purgative, was studied through various standardization parameters. Several tests and analytical techniques such as organoleptic evaluations (colour, odour, and taste), microscopy, physico-chemical evaluations (moisture content, total ash, acid insoluble ash, pH values, water, ethanol and hexane soluble extractives) and HPTLC fingerprinting of the formulation were carried out for its easy identification. The evaluation of contaminants such as heavy metals, aflatoxins, pesticide residues, and microbial load were also carried out to determine the presence of any toxins in the formulation. The data evolved in the present work will help in developing pharmacopoeial standards of Qurs-e-Mulaiyin.

KEYWORDS: Physico-chemical analysis, Qurs-e-Mulaiyin, Pharmacopoeial standards, Pharmacognosy, HPTLC fingerprinting.

INTRODUCTION

Laxatives can have tremendous effects on our digestive health. They can help relieve constipation and promote regular bowel movements. The Unani System of Medicine includes a plethora of traditional formulations including a number of laxatives. Qurs-e-Mulaiyin is a popular laxative which is safe if consumed even for longer time. It is also considered to be a non-habit-forming drug. Apart from its use as a laxative, it is also beneficial in stomachache and chronic headache.[1] Qurs-e-Mulaiyin is mentioned in the National Formulary of Unani Medicine Part-I under the category of Aqras.[2] Qurs (Plural: Aqras) is an Arabic word which means flattened disc. Qurs-e-Mulaiyin is composed of ten different ingredients (Table 1) which individually have magnificent medicinal properties. The ingredients like Saqmonia, Rewand Chini and Turbud are well known for their laxative or purgative properties. Ustukhuddus is stomachic and relieves flatulence. It also removes morbid humors from the body [3]. Badiyan and Mastagi are also used for relieving flatulence and stomachache. Post-e-Halela Kabuli, Post-e-Balela, Aamla and Halela Siyah are prescribed for strengthening the stomach.[3]

The present research work aims to develop quality parameters and evaluate the data to lay down the pharmacopoeial standards of Unani formulation Qurs-e-Mulaiyin. The conventional parameters such as organoleptic evaluations, microscopy, physico-chemical evaluations such as water, alcohol and hexane soluble extractives, total ash, acid insoluble ash, moisture content, pH values were carried out along with HPTLC fingerprinting. The WHO quality control parameters such as heavy metals estimation, aflatoxins, microbial load and pesticide estimation were also analyzed in order to assess the quality of the formulation.

MATERIALS AND METHODS

Preparation of Formulation

All the ingredients were procured from local raw drug dealer and were identified botanically using pharmacognostical methods.[4,5] The ingredients were further validated by comparing with the monographs available in UPI (Part I), Vol. I, II, IV & V.[6]

All the ingredients were taken of pharmacopoeial quality. The ingredients were cleaned and dried under shade to remove the moisture if any. The ingredients no.

1-10 [Table 1] were crushed separately in an iron mortar to obtain their coarse powders. The coarse powders were further ground in a grinder to get their fine forms. The fine powders were mixed together thoroughly and sieved through mesh no. 80. Then sufficient quantity of water was added to the mixture and again mixed thoroughly to obtain the *lubdi* (mass). The aqras were prepared from the *lubdi* (mass) by mechanical process and dried under shade. The prepared drug was stored in tightly closed glass container free from moisture and kept in a cool and dry place. The drug was prepared in three batches.

Table 1: Formulation composition.

S. No.	Ingredients	Botanical Name/English Name	Part used	Form	Qty.
1	Badiyan	*Foeniculum vulgare* Mill.	Fruit	Powder	50g
2	Mastagi	*Pistacia lentiscus* L.	Resin	Powder	50g
3	Ustukhuddus	*Lavendula stoechas* L.	Inflorescence	Powder	50g
4	Saqmonia	*Convovulus scammonia* L.	Resin	Powder	50g
5	Rewand Chini	*Rheum emodi* Wall	Root	Powder	50g
6	Post-e-Halela Kabuli	*Terminalia chebula* Retz	Pericarp	Powder	20g
7	Post-e-Balela	*Terminalia bellerica* Roxb.	Pericarp	Powder	20g
8	Aamla	*Embellica officinalis* Gaertn	Fruit	Powder	20g
9	Halela Siyah	*Terminalia chebula* Retz	Fruit	Powder	20g
10	Turbud	*Operculina turpethum* L. Silva Manso	Fruit	Powder	20g

Microscopy
5g of the powdered drug was taken and stirred gently with hot water in a beaker. The supernatant was discarded and the residue was washed with distilled water. A little residue was stained with iodine solution and mounted in 50% glycerin. Some of the residue was heated in chloral hydrate solution and mounted in 50% glycerin and a little residue was boiled in 2% potassium hydroxide solution, washed with distilled water and mounted in 50% glycerin.[4,5,7]

Physico-chemical analysis
The physico-chemical parameters of Qurs-e-Mulaiyin viz. moisture content, extractive values (solubility in water, ethanol and hexane), ash values (total ash and acid insoluble ash) and pH values (1% and 10% aqueous solution) and volatile oil estimation were analyzed as per standard methods.[8,9]

High performance thin layer chromatography
The drug samples (2g each) were extracted separately with 25 ml each of chloroform and ethanol by sonicating for 20 minutes and filtered. The extracts were concentrated; made up to 10ml in volumetric flasks and used for HPTLC fingerprinting. 6µl of each extract was applied on aluminum TLC plate pre-coated with silica gel 60 F_{254} (E. Merck) by employing CAMAG Linomat IV automatic sample applicator. The plate was developed up to a distance of 9cm in twin trough glass chamber (10x10), using 10ml of the solvent system Toluene: Ethyl acetate: Formic acid (9:1:0.5) as mobile phase. The plate was air-dried at room temperature and observed under UV at the wavelength 254nm and 366nm. Further the plate was dipped in 1% vanillin-sulphuric acid reagent and heated at 105° C till coloured bands appeared. The plate was finally examined under visible light.[13 – 15]

Quality control analysis
The herbal medicines including Unani medicines are being used worldwide as effective remedies which have lesser side effects. People's faith in Unani medicines leads to concern over their quality assurance. So, the different quality control parameters like microbial load, heavy metals, aflatoxins and pesticide residues were carried out to verify the required quality of the formulation Qurs-e-Mulaiyin. Estimation of microbial load was conducted as per standard method.[10] Aflatoxins and heavy metal analysis were carried out by respective use of HPLC (Thermo Fisher)[10] and Atomic Absorption Spectrophotometer (LABINDIA).[11] Pesticide residues were analyzed using GC-MS system (Agilent) equipped with mass selective detector as per standard methods.[11,12]

RESULTS AND DISCUSSION
Macroscopic description
Qurs-e-Mulaiyin is a dark brown tablet with pleasant odour and mucilaginous taste.

Microscopic observation
The drug did not show any filth, fungus or objectionable matter while the sample was spread in a petri dish. (Fig. 1).

Fig. 1: Qurs-e-Mulaiyin.

The microscopy shows fragment of epicarp and vittae in surface view (Badiyan); tufted hairs of various kind (Ustukhuddus); thin walled parenchyma cells containing abundant starch grains and rosette crystals of calcium oxalate, pieces of vessels with scleriform thickenings (Rewand Chini); group of fibers and pitted scleroids present mostly in groups (Post-e-Halela Kabuli); uniseriate elongated trichenes and group of elongated sclerotic cells (Post-e-Balela); group of thin walled endoscopic parenchyma cells and sclereids in groups with lumen and pitted walls (Aamla); pieces of elongated lignified fibers present mostly in group and groups of sclerids (Halela Siyah); parenchyma cells filled with abundant simple, spherical starch grains, fragment of cork cells in surface view and pieces of pitted vessels (Turbud). (Fig. 2).
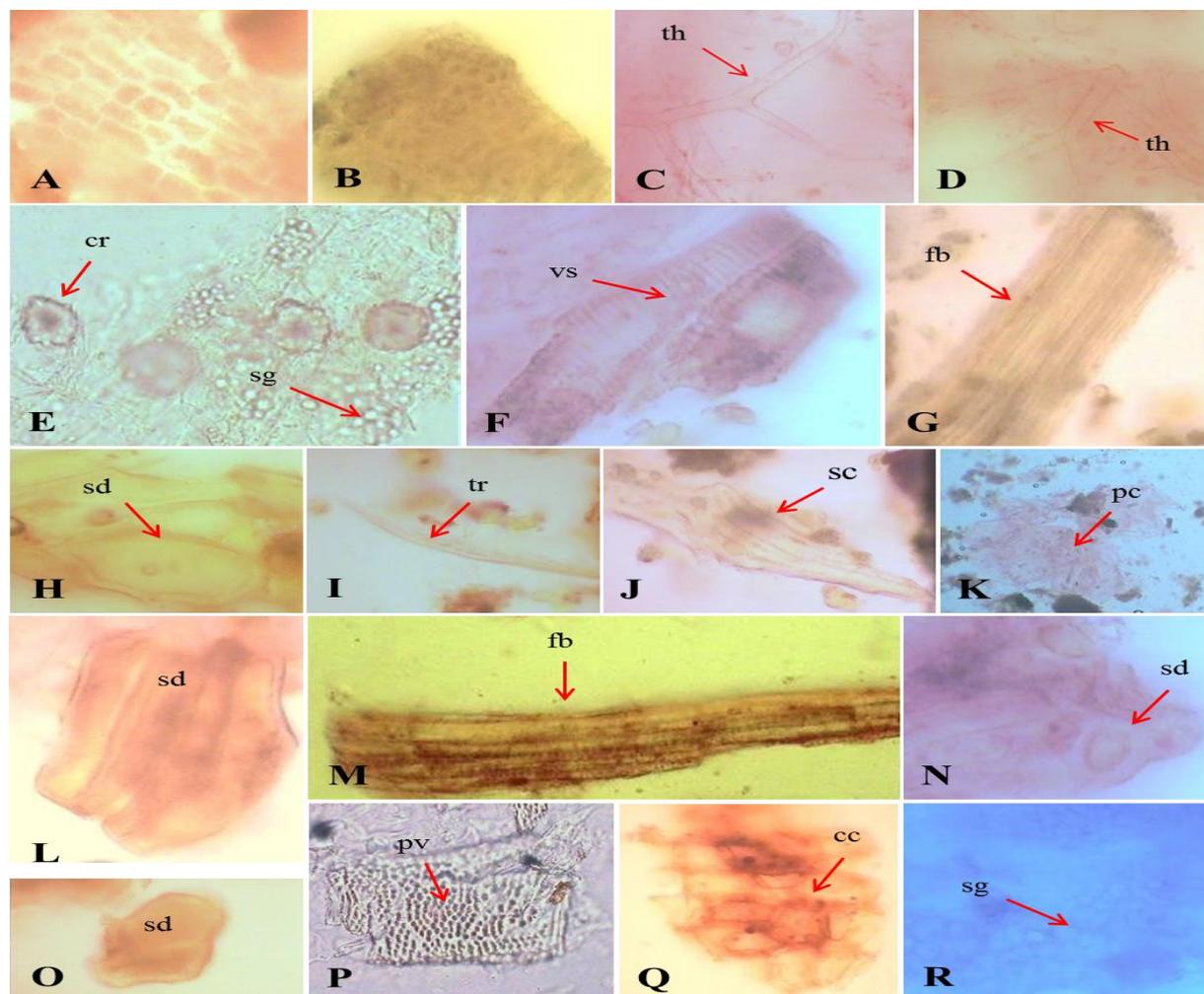

Fig. 2 (A-R): Microscopic examination of different types of cells in Qurs-e-Mulaiyin.

(A) Surface view of epicarp cells of badiyan (40x); (B) Fragment of vittae cells of badiyan (40x); (C-D) Tufted hair (th) cells of Ustukhuddus (40x); (E) Parenchyma cells (pc) of Rewandchini with starch grains (sg) and calcium oxalate crystals (cr) (40x); (F) Vessel cells (vs) of Rewandchini (40x); (G) Group of fiber cells (fb) of Post-e-Halela Kabli (40x); (H) Group of sclereid cells (sd) of Post-e-Halela Kabli (40x); (I) Trichome cells (tr) of Post-e-Balela (40x); (J) Sclerotic cells (sc) of Post-e-Balela (40x); (K) Mesocarpic parenchyma cells (pc) of Aamla (10x); (L) Group of sclereid cells (sd) of Aamla (40x); (M) Group of fibers cells (fb) of Halela Siyah (40x); (N-O) Sclereid cells (sd) of Halela Siyah (40x); (P) Pitted vessels (pv) of Turbud (40x); (Q) Surface view of cork cells (cc) in Turbud (40x); (R) Parenchyma cells with starch grains (sg) of Turbud (40x).

Physico-chemical analysis
The physico-chemical data of the drug Qurs-e-Mulaiyin are shown in Table 2. The extractive values show that the solubility of phyto-constituents of the drug was more in Ethanol (36.86%) in comparison to water (27.93%) and Hexane (13.88%). The moisture content in drug was low as the loss in weight on drying at 105^0C occurred below 10%. Quantitative standards reveal the presence of negligible amount of siliceous matter in the sample as the total ash and acid insoluble ash was found to be 4.34% and 1.31% respectively. The aqueous extract of the drug was slightly acidic as pH falls in the range of 4-5.

Quality control parameters
Microbial load
Estimation of microbial growth is very important parameter in traditional medicines. It indicates whether the drug contains disease causing and spoilage micro-organisms in permissible limits. The assessment is done for evaluating the total bacterial count, total fungal count, count of bacteria belonging to the *Enterobacteriaceae* family, count of pathogens like *E. coli, Staphylococcus aureus*, *Salmonella spp.* and *Pseudomonas aeruginosa*. The results of microbial load is shown in Table 3 which indicate that the drug is safe for internal use.

Aflatoxins
The results of aflatoxins analysis of the drug are given in Table 4. Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus, A. parasiticus* and *A. nomius*. The results do not show the presence of any of the aflatoxins contents (B1, B2, G1, and G2) in Qurs-e-Mulaiyin.

Pesticide residues
The results of pesticide residues are given in table 5. Harvest of herbal material without the use of pesticides is very difficult due to several factors. But as per WHO guidelines, the major concern is whether the drug contains pesticide residue in permissible limits or not. In order to estimate the pesticide residue, the drug was analyzed on GC-MS. The results indicated that the drug is free of pesticide residues and safe for use.

Heavy metal analysis
The results of Heavy metal estimation are given in Table 6. Heavy metals are hazardous to human health and may cause many fatal diseases. A heavy metal has relatively high density or atomic weight and is toxic or poisonous even at low concentrations. The heavy metal content in Qurs-e-Mulaiyin was found to be below detection limit which indicated that the drug was free from heavy metal contamination.

HPTLC Profile
HPTLC fingerprinting is sensitive, reliable and convenient tool for identification of crude drugs as well as complex compound formulations as the plant species produce distinct chromatograms. HPTLC images of both the extracts of Qurs-e-Mulaiyin were observed under UV 254nm, UV 366nm and under visible light after derivatization. All the batches of Qurs-e-Mulaiyin show similar colorful bands with similar R_f values. Moreover, their densitograms are almost superimposed on each other. This shows batch-to-batch consistency of the formulation. (Fig. 3 - 6).

BI BII BIII
UV 254nm
(a)

BI BII BIII
UV 366nm
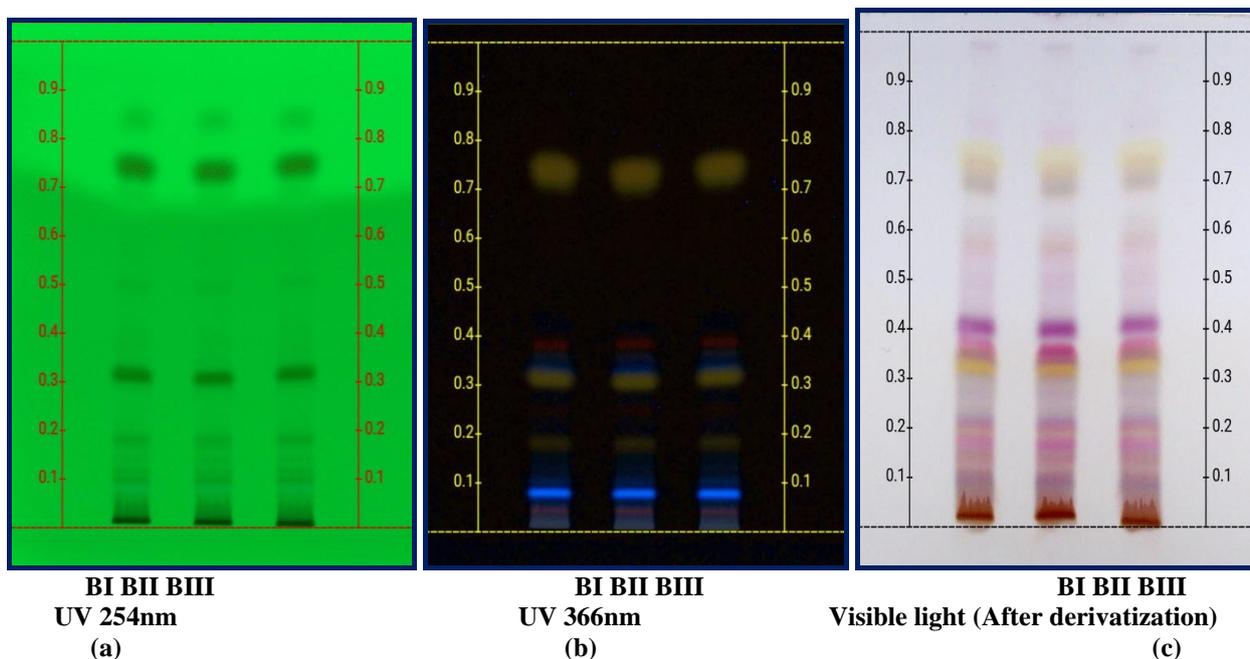**(b)**

BI BII BIII
Visible light (After derivatization)
(c)

Fig. 3: HPTLC Images of Chloroform extracts.
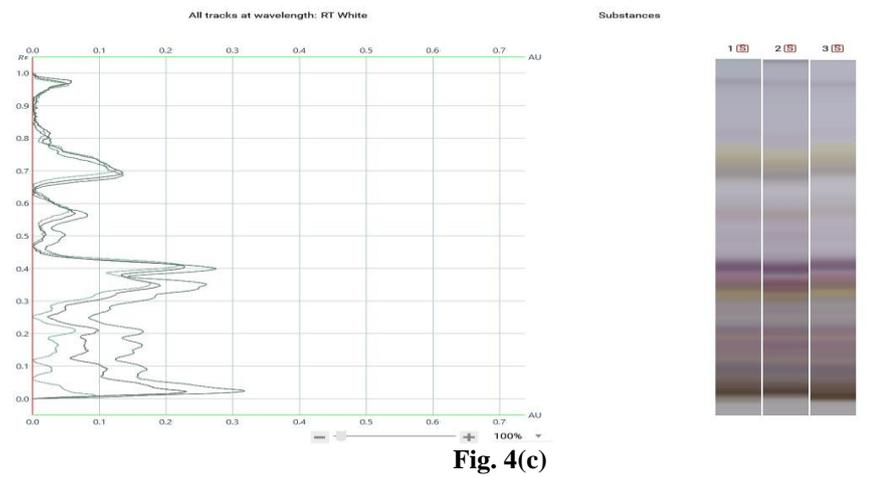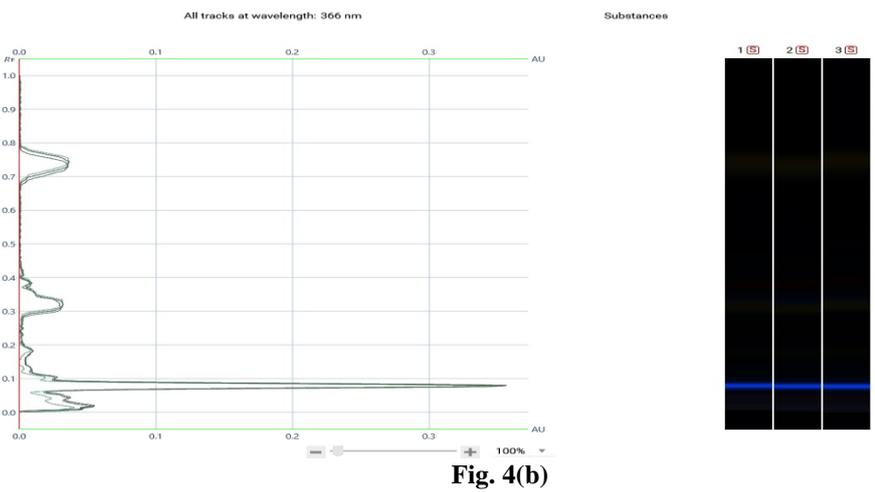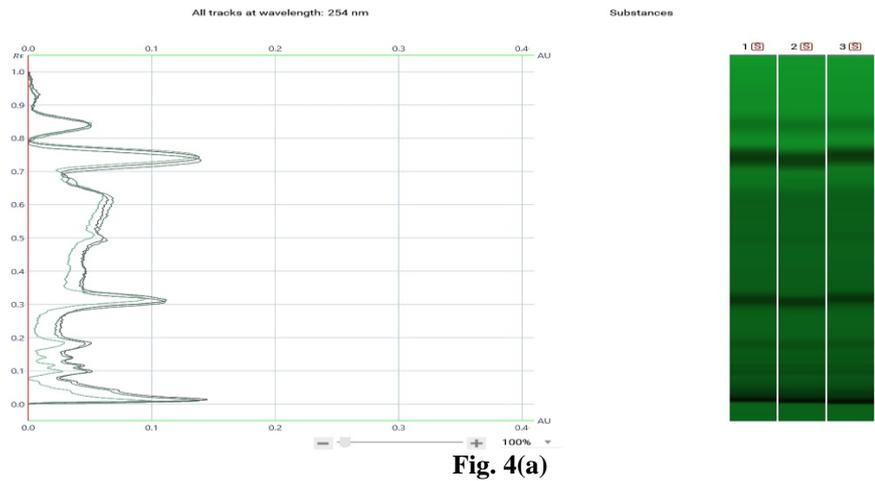
Fig. 4(a)

Fig. 4(b)

Fig. 4(c)

Fig. 4: HPTLC Densitometry chromatogram of three batches of chloroform extracts.

BI BII BIII
UV 254nm
(a)

BI BII BIII
UV 366nm
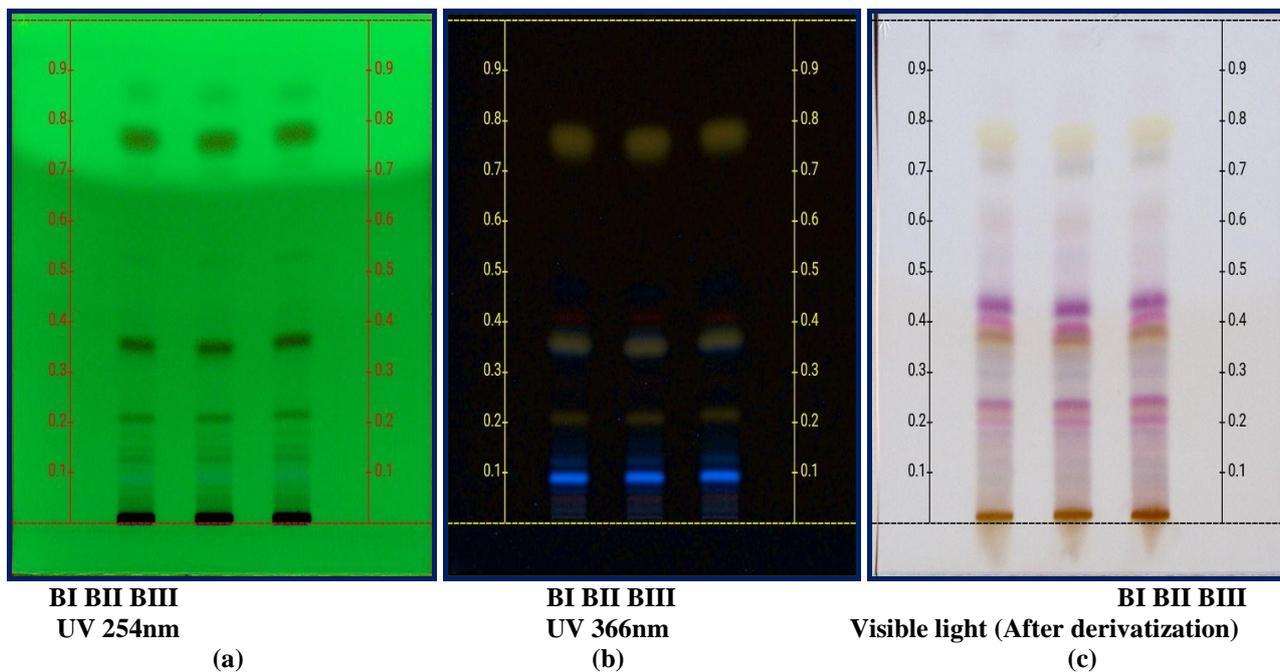**(b)**

BI BII BIII
Visible light (After derivatization)
(c)

Fig. 5: HPTLC Images of Ethanol extracts.
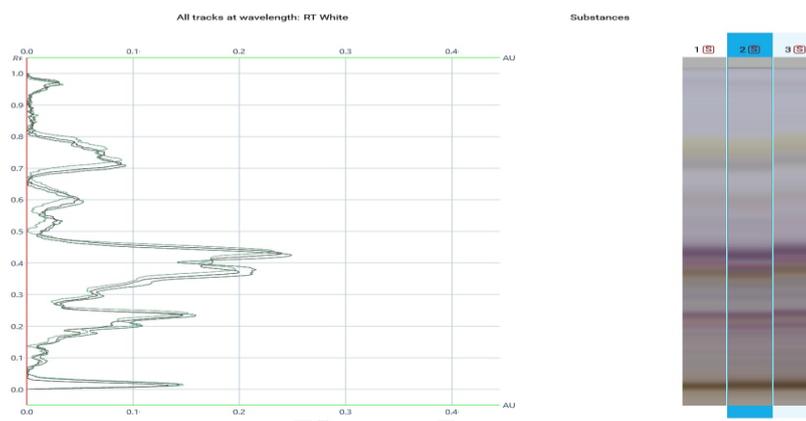

Fig.6(a)

Fig.6(b)
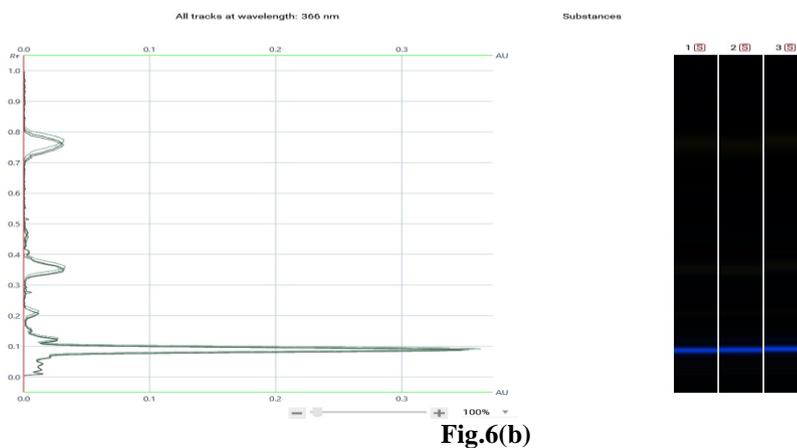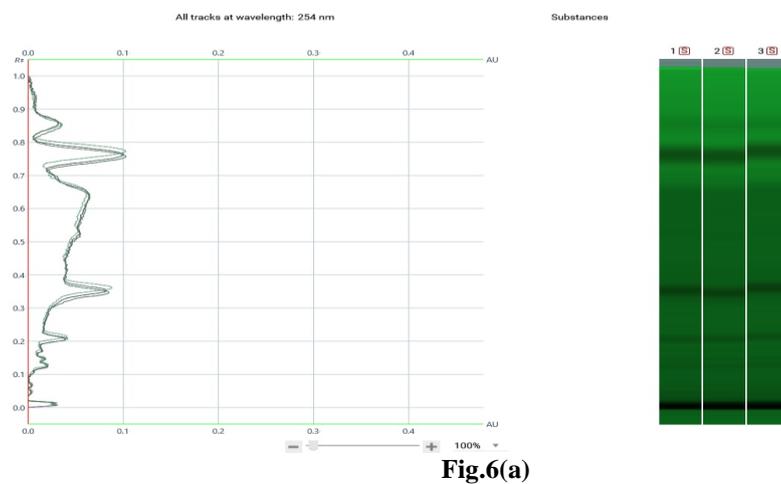
Fig.6(c)
Fig. 6: HPTLC Densitometry chromatogram of three batches of Ethanol extracts.

Table 2: Physico-chemical parameters.

S. No.	Parameters	Results
1	Water soluble extractive (%)	35.03-36.86
2	Alcohol soluble extractive (%)	26.11-27.93
3	Hexane soluble extractive (%)	12.25-13.88
4	Loss in wt. on drying at 105^0C	6.72-7.85
5	Total ash (%)	4.03-4.34
6	Acid Insoluble ash (%)	1.12-1.31
7	pH of 1% aqueous Soln.	5.14-5.63
8	pH of 10% aqueous Soln.	4.40-4.90
9	Volatile oil	Traces

Table 3: Microbial load.

S. No.	Parameter Analyzed	Results	WHO Permissible limit
1	Total *Bacterial* load	6×10^1 cfu / g	10^5 cfu / g
2	Total *Fungal* count	< 1 cfu/ g	10^3 cfu / g
3	Enterobacteriaceae	Absent	Nil
4	*Escherichia coli*	Absent	Nil
5	*Salmonella spp.*	Absent	Nil
6	*Staphylococcus aureus*	Absent	Nil
7	*Pseudomonas aeruginosa*	Absent	Nil

Table 4: Aflatoxins level.

S. No.	Parameter Analyzed	Results	WHO Permissible limit
1	B1	Not detected	< 2ppb
2	+B2+G1+G2	Not detected	< 5ppb

Table 5: Pesticide residue.

S. No.	Pesticide name	Results	WHO Permissible limit (mg/kg)
1	Alachor	BLQ	0.02
2	Aldrin	BLQ	0.05
3	Azinphos –methyl	BLQ	1.0
4	Chlordane (cis & trans)	BLQ	0.05
5	Chlorfenvinphos	BLQ	0.5
6	Chlorpyrifos	BLQ	0.2
7	Chlorpyrifos-methyl	BLQ	0.1
8	Cypermethrin	BLQ	1.0
9	DDT	BLQ	1.0
10	Deltamethrin	BLQ	0.5
11	Diazinon	BLQ	0.5
12	Dichlorvos	BLQ	1.0
13	Dimethoate	BLQ	0.1

14	Dieldrin	BLQ	0.03
15	Endosulphan	BLQ	3.0
16	Endrin	BLQ	0.05
17	Ethion	BLQ	2.0
18	Fenitrothion	BLQ	0.5
19	Fenvalerate	BLQ	1.5
20	Heptachlor	BLQ	0.05
21	Hexacholobenzene	BLQ	0.06
22	Lindane (gamma HCH)	BLQ	0.6
23	Malathion	BLQ	1.0
24	Parathion	BLQ	0.5
25	Parathion-methyl	BLQ	0.2
26	Permethrin	BLQ	1.0
27	Phosalone	BLQ	0.1
28	Primiphos methyl	BLQ	0.1

*BLQ-Below Limit of Quantification

Table 6: Heavy metals.

S. No.	Parameter Analyzed	Results	WHO Permissible limit (ppm)
1	Lead	Not detected	10.00
2	cadmium	Not detected	0.30
3	Arsenic	Not detected	3.00
4	Mercury	Not detected	01.00

CONCLUSION

It can be concluded that organoleptic parameters are not much reliable in identification of herbal drugs as the ingredients are usually powdered and mixed together for preparation of compound formulations. The present study therefore holds high significance as the microscopic features; various physico-chemical parameters, HPTLC profile etc. provide criteria for easy identification of the formulation Qurs-e-Mulaiyin and quality control analysis ensures the authenticity, quality and efficacy of the medicine.

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