Standardization and HPTLC Fingerprinting of unani compound formulation Habb-E-Muqil Jadeed

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ABSTRACT:
Standardization and evaluation of Unani formulations is very much essential to ascertain the quality of medicine. Unani medicines have played a significant role in maintaining the good health among the masses around the globe. Unani medicines are accepted as important therapeutic agents for treatment of various kinds of diseases, because of its harmonious nature with the human body. Habb-e-Muqil Jadeed a Unani Compound formulation is frequently prescribed for the treatment of Kabz (Constipation) in Unani system of medicine. In many instances, it has been noticed that due to adulterated raw herbs it is very difficult to find the genuine product in market place. The present communication deals with the proper authentification, identification, determination of physico-chemical constants, chromatographic profile, aflatoxin contamination, pesticide residue and heavy metal analysis of the formulation. The present standardization and HPTLC fingerprinting developing sets the standard for quality assurance of the formulation in the global marketing.

KEYWORDS: Kabz (Constipation), Aflatoxin contamination, pesticide residue, chromatographic profile.

INTRODUCTION:

Habb are small, rounded and uniformly shaped medicinal preparations used in Unani System of Medicine[1]. Habb-e-Muqil Jadeed was frequently prescribed by unani physician in the treatment of Kabz (Constipation)[2]. The composition of Habb-e-Muqil Jadeed was mentioned in NFUM consisting of ingredients as given in Table 1. Standardization of herbal drugs is an essential need for the identity, purity and strength determination and also to ascertain the quality control of the drugs in batch to batch variation[3-9].

MATERIAL AND METHODS:

The raw drug ingredients of the Habb-e-Muqil Jadeed (figure 1) were procured form the Pharmacy of NRIUMSD, Hyderabad. All the ingredients (figure 2) are identified by expert Botanist using pharmacopoeial standards[10]. The physico-chemical studies of the Habb-e-Muqil Jadeed were carried out as per the methods described in the Unani Pharmacopoeia of India, for HPTLC profile Desaga sarstedt Gruppe system with automatic sample applicator was used and photographs were taken with the help of Desaga photo-documentation system[11,12]. Microbial contamination, aflatoxin contamination, Heavy metal estimation [13, 14] pesticide residue were studied as per UPI.[17]

FIG.1: HABB-E-MUQIL JADEED

FIG.2: RASAUT

FIG.3: ARAROT

FIG.4: MUQIL AJRAQ

FIG.5: SAT PUDINA

TABLE 1. FORMULATION COMPOSITION [15]

S.No	Name	Botanical/sci. Name	Part used	Qty.
1.	Muqil Azraq[16]	*Commiphora mukul* (Hook ex Stocks) Engl.	Oleo-gum resin	250gm
2.	Rasuat Zard[17]	*Berberis aristata* Dc.	Extract	280gm
3.	Satt-e-Podina [18]	*Mentha arvensis* Linn.	Purified extract	5gm
4.	Ararot[19,20]	*Maranta arundinaceae* Linn.	Starch	Q.S.
5.	Roughan-e-Kunjad [17]	Oil of *Sesamum indicum* Linn.	Oil	Q.S.
6.	Aab-e-Adrak[21]	*Zingiber officinale* Rosc.	water	Q.S.

Method of preparation:

It is prepared according to the composition of the formulation composition given above. All the ingredients were taken of pharmacopoeial quality and cleaned by removing the foreign materials. Muqil Azraq and Rasuat Zard are purified and powdered. Satt-e-Podina was added to the powered mixture. The mixture so obtained was rubbed (charb) in Raughan-e-Kunjad. Ararot Paste was made using Aab-e-Adrak. All ingredients were mixed and passed through mesh no. 40 to make the granules. Granules are subjected to make the Habb (Tablets).

Physico-chemical analysis

The Physico-chemical parameters were carried out for the formulation Habb-e-Muqil Jadeed i.e., total ash, acid insoluble ash, solubility in water and alcohol, loss in weight on drying at 105 ^{0}C as per the Standard operating procedures mentioned in the Unani Pharmacopoeia of India[17, 22-26].

Thin layer chromatography and HPTLC[27-35]

Two gram of drug sample was extracted with 20 ml of Petroleum ether (40-60 ^{0}C) by refluxing on a water bath for 30 min. The extract so obtained was filtered and concentrated up to 5 ml to carry out the thin layer chromatography. Concentrated Petroleum ether extract was applied on TLC plate.

RESULT AND DISCUSSION:

Analytical Profile

Organoleptic characters

Habb-e-Muqil Jadeed is dark brown in colour, solid, mint like odour and slightly bitter in taste. The surface of the tablet is smooth as shown in the figure1. Organoleptic evaluations are carried out based on the method described by Siddique et al[36].

Physico-chemical analysis

The Physico-chemical parameters of the formulations Habb-e-Muqil Jadeed were studied such as total ash, acid insoluble ash, solubility in water and alcohol, loss in weight on drying at 105 ^{0}C, and the results are tabulated in table 2. Safety evaluation parameters such as microbial load, aflatoxins, heavy metals and pesticide residues were studied as per method described in WHO guidelines[13,37-38].

TABLE 2. PHYSICO CHEMICAL PARAMETERS OF UNANI COMPOUND FORMULATION HABB-E-MUQIL JADEED

S.No	Parameter	Sample-I	Sample-II	Sample-III
1.	Colour	Dark Brown	Dark Brown	Dark Brown
2.	Odour	mint like	mint like	mint like
3.	Taste	slightly bitter	slightly bitter	slightly bitter
4.	Total ash (*%w/w*)	25.6728	26.1026	26.3256
5.	Acid insoluble ash (*%w/w*)	22.4625	22.3562	22.4126
6.	Alcohol soluble matter (*%w/w*)	17.2632	17.1706	17.4281
7.	Water soluble matter (*%w/w*)	34.2736	34.3688	34.2876
8.	Loss in wt. on drying at 105 ^{0}C (*%w/w*)	5.0016	5.0156	5.0562
9.	pH of 1% aqueous solution	5.70	5.74	5.72
10.	pH of 10% aqueous solution	5.65	5.69	5.67

Safety evaluation

Safety evaluation such as heavy metal analysis, microbial load estimation, aflatoxin contamination, and pesticide residue analysis were analyzed and the results are tabulated in the tables 3-6.

TABLE 3: HEAVY METAL ANALYSIS (METHOD OF TESTING AS PER WHO[13] AND AOAC[14])

S.No.	Parameter analyzed	Results			WHO permissible limit
		Batch 1	Batch 2	Batch 3	
1	Lead (Pb)	ND	ND	ND	10 ppm
2	Cadmium(Cd)	ND	ND	ND	0.3 ppm
3	Arsenic (As)	ND	ND	ND	3.0 ppm
4	Mercury (Hg)	ND	ND	ND	1.0 ppm

TABLE 4: MICROBIAL CONTAMINATION

S.No.	Parameter analyzed	Results	WHO permissible limit
1	Total Bacterial load	$25x10^2$	not more than 10^5/g
2	Salmonella spp.	Nil	Nil
3	Escherichia coli	Nil	Nil
4	Total Fungal count	10x10	not more than 10^3/g

TABLE 5: AFLATOXIN CONTAMINATION

S.No.	Parameter analyzed	Results	WHO permissible limit
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

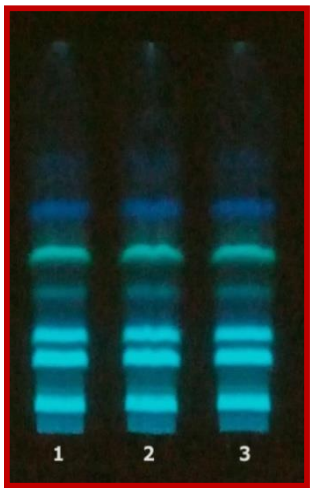
TABLE 6: PESTICIDE RESIDUE: (METHOD OF TESTING AOAC 2007.01 BY GC MSMS / LC MSMS)[14]

S. No	Test parameters	Results (mg/kg)
1	Aldrin	BLQ (LOQ-0.01)
2	Chlordane (*cis* & *trans*)	BLQ (LOQ-0.01)
3	Alachlor	BLQ (LOQ-0.01)
4	Azinphos-methyl	BLQ (LOQ-0.01)
5	Chlorfenviniphos	BLQ (LOQ-0.01)
6	Endosulphan (all isomers)	BLQ (LOQ-0.01)
7	Endrin	BLQ (LOQ-0.01)
8	Chlorpyrifos	BLQ (LOQ-0.01)
9	Chlorpyrifos-methyl	BLQ (LOQ-0.01)
10	Cypermethrin	BLQ (LOQ-0.01)
11	DDT	BLQ (LOQ-0.01)
12	Deltamethrin	BLQ (LOQ-0.01)
13	Diazinon	BLQ (LOQ-0.01)
14	Dichlorvos	BLQ (LOQ-0.01)
15	Ethion	BLQ (LOQ-0.01)
16	Fenitrothion	BLQ (LOQ-0.01)
17	Fenvalerate	BLQ (LOQ-0.01)
18	Heptachlor	BLQ (LOQ-0.01)
19	Hexachlorobenzene	BLQ (LOQ-0.01)
20	Lindane(gamma-HCH)	BLQ (LOQ-0.01)
21	Malathion	BLQ (LOQ-0.01)
22	Parathion	BLQ (LOQ-0.01)
23	Parathion methyl	BLQ (LOQ-0.01)
24	Permethrin	BLQ (LOQ-0.01)
25	Phosalone	BLQ (LOQ-0.01)
26	Pirimiphos methyl	BLQ (LOQ-0.01)

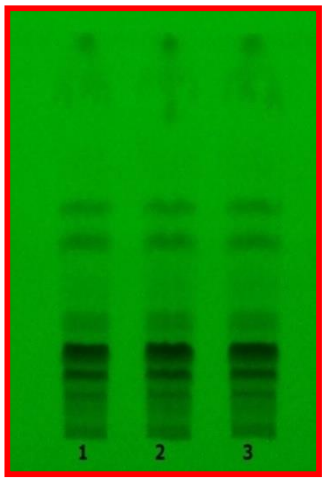
**BLQ- Below limit of Quantification / LOQ-limit of quantification*

Thin layer chromatography

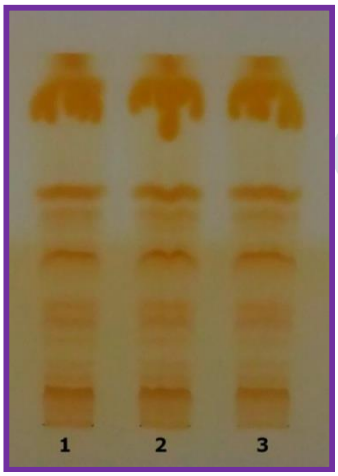
The TLC studies of Petroleum ether extract are tabulated in table 7. Petroleum ether (40-60 ^{0}C) extract was spotted on silica gel "G" plate and developed with Toluene: Ethyl acetate (9: 1) as mobile phase shows eight spots under UV 366nm at R_f values 0.03 (Fluorescent Blue), 0.06 (Fluorescent Blue), 0.31 (Fluorescent Blue), 0.39 (Fluorescent Blue), 0.49(Fluorescent Blue), 0.60(Fluorescent Blue), 0.69(Blue), and 0.82 (blue); and under UV 254nm shows seven spots at R_f values 0.11, 0.17, 0.21, 0.29, 0.46, 0.60, 0.90 (All black); and under Iodine vapours shows seven spots at R_f values 0.11, 0.29, 0.33, 0.49, 0.60, 0.64, 0.90 (All brown); and under visible region after derivatizing with anisaldehyde sulphuric acid shows ten spots at R_f values 0.06(Grey), 0.11(brown), 0.29(reddish pink), 0.33(Grey), 0.49(blue), 0.60(Purple), 0.63(Purple), 0.67(Dark Purple), 0.90(Brown) and 0.99 (Purple) as shown in the figure 3.



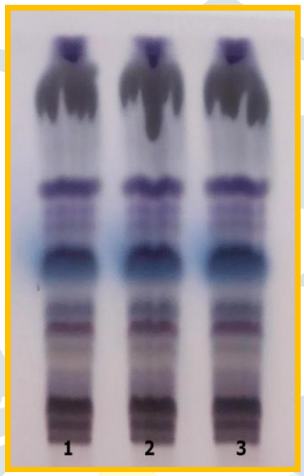
At UV 366nm



At UV 254nm



Expose to Iodine vapours



After derivatization with anisaldehyde sulphuric acid

FIG. 3: HPTLC OF PETROLEUM ETHER EXTRACT OF HABB-E-MUQIL JADEED

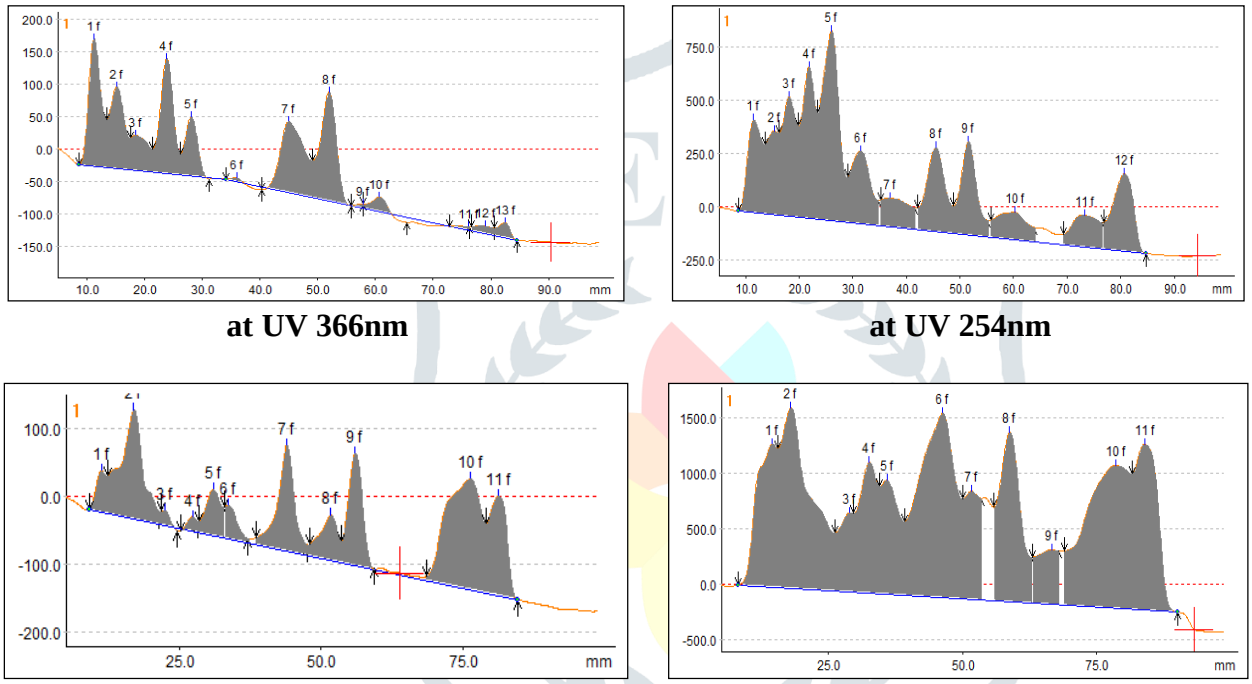
TABLE 7: RF VALUES OF PETROLEUM ETHER (40-60 ^{0}C) EXTRACT.

Solvent System	R_f Values			
	Uv-366 nm 8 Spots	**UV-254 nm 7 Spots**	**Under Iodine Vapour 7 Spots**	**under visible region after derivatizing with anisaldehyde sulphuric acid 10 Spots**
Toluene : Ethyl acetate (9:1)	0.03 (Fluorescent Blue)	0.11 (Black)	0.11 (Brown)	0.06 (Grey)
	0.06 (Fluorescent Blue)	0.17 (Black)	0.29 (Brown)	0.11 (brown)
	0.31 (Fluorescent Blue)	0.21 (Black)	0.33 (Brown)	0.29 (reddish pink)
	0.39 (Fluorescent Blue)	0.29 (Black)	0.49 (Brown)	0.33 (Grey)
	0.49 (Fluorescent Blue)	0.46 (Black)	0.60 (Brown)	0.49 (blue)
	0.60 (Fluorescent Blue)	0.60 (Black)	0.64 (Brown)	0.60 (Purple)

	0.69 (Blue)	0.90 (Black)	0.90 (Brown)	0.63 (Purple)
	0.82 (Blue)			0.67 (Dark Purple)
				0.90 (Brown)
				0.99 (Purple)

HPTLC analysis

Petroleum ether (40-60 ^{0}C) extract was spotted on silica gel "G" plate and developed with toluene: ethyl acetate (9: 1). The TLC plate under various detection systems such as UV 366nm, UV 254nm, upon exposure to iodine vapours and after derivatization with anisaldehyde sulphuric acid and heating at 105 ^{0}C was subjected to scanning using the HPTLC densitometer. The corresponding densitograms were obtained in which peaks for the components are appeared as shown in the figure 4. The corresponding peak areas obtained in various detection systems were recorded and depicted in the tables 8-11.



at UV 366nm **at UV 254nm**

upon exposure to Iodine vapours at 580 nm **after derivatization with anisaldehyde sulphuric acid at 580 nm**

FIG. 4: DENSITOGRAM OF PETROLEUM ETHER EXTRACT OF HABB-E-MUQIL JADEED

TABLE 8: PEAK LIST OF PETROLEUM ETHER EXTRACT OF HABB-E-MUQIL JADEED AT UV 366NM (FIG-4)

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	11.3	476.44	16.39	192.94	0.03
2	15.3	364.37	12.54	123.73	0.09
3	18.5	179.06	6.16	53.61	0.13
4	23.9	463.41	15.94	173.88	0.20
5	28.1	217.77	7.49	88.76	0.26
6	36.0	9.22	0.32	5.19	0.36
7	45.0	509.79	17.54	107.26	0.49
8	52.0	519.30	17.87	163.88	0.58
9	57.8	5.94	0.20	5.27	0.66
10	60.7	61.73	2.12	21.60	0.70
11	76.1	9.48	0.33	4.85	0.91

12	79.1	38.88	1.34	11.14	0.95
13	82.4	51.18	1.76	22.46	0.99

TABLE 9: PEAK LIST OF PETROLEUM ETHER EXTRACT OF HABB-E-MUQIL JADEED AT UV 254 NM

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	11.5	1334.12	7.46	432.94	0.03
2	15.4	923.35	5.17	390.65	0.09
3	18.1	1676.90	9.38	557.58	0.12
4	21.8	2016.44	11.28	706.49	0.17
5	26.0	3287.47	18.39	884.54	0.23
6	31.5	1423.97	7.97	338.97	0.30
7	36.9	779.78	4.36	134.16	0.38
8	45.5	1596.66	8.93	391.90	0.49
9	51.6	1520.32	8.50	436.35	0.58
10	60.2	842.18	4.71	129.38	0.69
11	73.3	872.99	4.88	148.06	0.87
12	80.7	1602.54	8.96	360.48	0.97

TABLE 10: PEAK LIST OF PETROLEUM ETHER EXTRACT OF HABB-E-MUQIL JADEED UPON EXPOSURE TO IODINE VAPOURS AT 580 NM

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	11.3	115.94	2.85	61.14	0.03
2	17.0	721.13	17.72	161.27	0.11
3	22.4	31.45	0.77	22.05	0.18
4	27.3	38.14	0.94	21.15	0.25
5	31.0	198.14	4.87	67.85	0.30
6	33.7	114.48	2.81	49.16	0.33
7	44.0	523.24	12.86	155.29	0.47
8	51.7	231.88	5.70	66.31	0.58
9	56.0	462.30	11.36	165.13	0.64
10	76.3	1128.60	27.73	162.80	0.91
11	81.3	504.28	12.39	146.60	0.98

TABLE 11: PEAK LIST OF PETROLEUM ETHER EXTRACT OF HABB-E-MUQIL JADEED AFTER DERIVATIZATION WITH ANISALDEHYDE SULPHURIC ACID AT 580NM

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	14.5	5526.42	7.81	1263.67	0.07
2	18.0	10439.06	14.75	1594.28	0.12
3	29.0	2045.95	2.89	698.94	0.26
4	32.6	4729.20	6.68	1155.83	0.31
5	35.9	3897.33	5.51	1005.83	0.35
6	46.3	12738.99	18.00	1631.12	0.49
7	51.7	3050.83	4.31	955.49	0.56
8	58.8	6845.14	9.67	1497.83	0.66
9	66.7	2182.80	3.08	481.58	0.76
10	78.6	12042.20	17.02	1261.55	0.92

11	84.0	7266.16	10.27	1464.47	0.99

CONCLUSION:

The present study had developed a standard of pharmacopoeial quality for the unani compound formulation Habb-e-Muqil Jadeed with respect to physico-chemical analysis, HPTLC analysis and safety evaluation such as heavy metal analysis, aflatoxin contamination, pesticide residue analysis and microbial load estimation. All the safety parameters were found within permissible limits as per WHO guidelines. Thus the present study serves as a reference standard for the study formation in future and also helps to check the quality control studies.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest

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