IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Qualitative Estimation Of A Unani Poly-Herbal Formulation Habb-E-Yarqaan

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Abstract : Unani System of medicine is a traditional healing system with a rich history and the drugs used in this system are derived from natural sources. Majority of them i.e., about 85% from plant origin and the rest are from animal & mineral origin. To produce the desired therapeutic effect in Unani system of medicine, the quality and efficacy of the formulation is very crucial. Present study deals to evaluate quality & efficacy of a polyherbal Unani formulation Habb-e-Yarqaan widely used in the treatment of Jaundice.

The present study was taken up to scientifically evaluate by the various physico-chemical parameters such as moisture content, loss on drying, extractive values such as water-soluble matter, ethanol soluble matter, percentage of ash values such as acid insoluble ash, water soluble ash and HPTLC analysis to identify various chemical components present in plant material. Evaluation of quality control parameters like heavy metal analysis, Microbial contamination, aflatoxin analysis and pesticide residue is also carried out in accordance with WHO guidelines such that the drug does not exceed the prescribed WHO limits for these parameter.

Index Terms - Polyherbal, Physico-chemical, HPTLC analysis, Quality control

1. Introduction

In the Unani system of medicine, jaundice is referred to as “Yarqaan” or “Kala-Pilia”. Unani medicine takes a holistic approach to healing, focusing on balancing the four humors (blood, phlegm, yellow bile and black bile) and restoring harmony to the body. Yarqaan (Jaundice) may be a quite common liver disorder. it's a condition during which an excessive amount of animal pigment is present within the blood. Unani medicine emphasizes identifying the root cause of the condition. Jaundice can result from various factors, such as liver disfunction, hepatitis or other disease. A Unani practitioner will carefully evaluate the patients symptoms and medical history to determine the cause of jaundice before recommending a treatment plan.

Yarqaan (Jaundice) is caused by varied reasons like backlog in canal that usually discharges digestive juice salts and pigment to the internal organ. The block within the digestive juice ducts will be because of gallstones or inflammation of liver, conjointly referred to as infectious disease. Jaundice may additionally be caused by excessive consumption of alcohol, cancer of exocrine gland anaemia and alternative diseases that have an effect on the liver like protozoan infection, infectious disease, typhoid fever and TB.

Symptoms of the Yarqaan (jaundice) are loss of appetite, nausea, yellow discoloration of the tongue, skin, eyes and body waste, extreme weakness, severe constipation and fever.

Habb-e-yarqaan is widely used in the treatment of Jaundice in Unani system of medicine and other Compound formulations prescribed are Arq-e- Biranjasif, Sharbat-e- Deenar, Majoon Dabeed-ul-Ward, Habb-e- Kabid Naushadri, Qurs-e- Jigar, Sharbat-e- Buzoori, Sharbat-e- Kasni and Arq-e Mako.

Habb-e-Yarqaan is a Unani poly-herbal formulation containing fifteen ingredients - namely Anisoon Roomi (Pimpinella anisum Linn., Dried ripe fruits), Tukhm-e-Turab (Raphanus sativus Linn, Seed), Tukhm-e-Kasoos (Cuscuta reflexa Roxb., Seed), Tukhm-e-Kahu (Lactuca sativa Linn., Seed), Tukhm-e-Kasni (Cichorium intybus Linn., Seed), Gul-e-Banafsha (Viola odorata Linn., Flower), Gul-e-Surkh (Rosa damascena Mill., Flower), Gul-e-Ghafis (Gentiana olivierii Griscb., Flower), Post-e-Halela-e-Zard (Terminalia chebula Retz., Fruit rind), Rewand-e-Chini (Rheum officianale Baillon, Dried rhizome), Naushadar (Ammonium Chloride, Salt), Sibr Zard (Aloe barbadensis Linn., Extract) , Barg-e-Kasni (Cichorium intybus Linn., Leaf), Barg-e-Kakronda (Blumea balsamifera Dc., Barg), Inab-us-salab (Solanum nigrum Linn., Whole plant). The present study is aimed to evaluate the parameters like physico-chemical analysis, HPTLC fingerprinting, Heavy metal analysis, Microbial load, Aflatoxins and Pesticide residue for identification, purification and also to ascertain the overall quality of the drug. Similar study has been published for other Unani compound formulations.[5,6,7,8,11]

2. MATERIAL AND METHODS

2.1 Collection of Materials

The raw materials were collected from National Research Institute of Unani Medicine for Skin Disorders (NRIUMSD) Hyderabad and are authenticated as per Pharmacopeial and official standards

2.2 Composition of formulation

Habb-e-Yarqaan is a tablet made with the ingredients in the formulation composition given in table1

Table-1: Formulation Composition[12]

S. No	Name	Botanical/ Scientific name[13]	Part
1.	Anisoon Roomi	Pimpinella anisum Linn.	Dried ripe fruits
2.	Tukhm-e-Turab	Raphanus sativus Linn	Seed
3.	Tukhm-e-Kasoos	Cuscuta reflexa Roxb.	Seed
4.	Tukhm-e-Kahu	Lactuca sativa Linn.	Seed
5.	Tukhm-e-Kasni	Cichorium intybus Linn.	Seed
6.	Gul-e-Banafsha	Viola odorata Linn.	Flower
7.	Gul-e-Surkh	Rosa damascena Mill.	Flower
8.	Gul-e-Ghafis	Gentiana olivierii Griscb.	Flower
9.	Post-e-Halela-e-Zard	Terminalia chebula Retz.	Fruit rind
10.	Rewand-e-Chini	Rheum officianale Baillon	Dried rhizome
11.	Naushadar	Ammonium Chloride	Salt
12.	Sibr Zard	Aloe barbadensis Linn.	Extract
13.	Barg-e-Kasni	Cichorium intybus Linn.	Leaf
14.	Barg-e-Kakronda	Blumea balsamifera Dc.	Barg
15.	Inab-us-salab	Solanum nigrum Linn.	Whole plant

2.3 Organoleptic Characters[2,4]**:** Organoleptic evaluation refers to assess the herbal formulation by smell, colour, odour and taste were carried out based on method, mentioned in Unani Pharmacopeia of India

2.4 Physiochemical analysis[1,2]

Physicochemical value such as the percentage of water-soluble matter, alcohol soluble matter, total ash, acid insoluble ash, loss on drying at 105 ^{0}C, pH of 1%solution and pH of 10% solution were calculated as per the Unani Pharmacopeia of India.

2.5 TLC/HPTLC finger printing analysis [10,14]

a. Preparation of extract of the sample drug

2 g of sample was extracted with 20 ml of Petroleum ether (40-600C) by refluxing on a water bath for 30 min. The extract so obtained was filtered and concentrated to 5 ml. The petroleum ether extract was used to carry out the thin layer chromatography.

b. Development and determination of the solvent system

Petroleum ether (40-600C) extract was spotted on silica Gel 60 F254 plate. After trying with various solvent system with variable volume ratio, the suitable solvent system Toluene: Ethyl Acetate (9: 1) as mobile phase was selected in its proportional ratio.

c. Detection system

After developing the TLC plate, it was dried at room temperature and the spots were observed at UV 366 nm, UV 254 nm, under Iodine chamber and under Visible region with anisaldehyde sulphuric acid to record the fingerprint spectrum.

d. HPTLC instrument condition

HPTLC was performed on 20 cm X 10 cm precoated Aluminium Sheets of Silica Gel 60 F254 (Merck). Sample solution about 10µl were applied as 10 mm width band using automatic TLC applicator system of the DESAGA Sarstedt Gruppe (Germany). A Linear ascending development with Toluene: Ethyl Acetate (9:1 v/v) as mobile phase was carried out in a twin through glass chamber previously saturated with mobile phase vapour for 20 min. at room temperature (25 ± 20 C) The development of solvent distance was 80 mm. After development plates were dried. TLC plate was scanned by densitometer of DESAGA Sarstedt Gruppe (Germany) at 366nm, 254 nm wavelength to record fingerprint spectrum.

2.6 Estimation of microbial load[9]

The estimation of microbial load viz. total bacterial count (TBC), total fungal count (TFC), Enterobacteriaceae, Escherichia coli, Salmonella spp. and Staphylococcus aureus were determined as per WHO, 1998.

2.7 Estimation of heavy metals [3,9]

The procedure used for the analysis of heavy metals like lead, cadmium, mercury and arsenic was as per WHO, 1998 and AOAC, 2005.

a. Instrument details and operating parameters

Thermo Fisher M Series, 650902 V1.27 Model Atomic Absorption Spectrometer (AAS) was used for the analysis.

Lead and cadmium

Instrument technique - Flame technique; wavelength (Lead) - 217 nm; wavelength (Cadmium) - 228.8 nm; slit width - 0. 5 mm; lamp current (Pb) - 4.0 mA; lamp current (Cd) - 3.0 mA; carrier gas and flow rate - air and acetylene, 1.1 L/min; sample flow rate - 2 ml/min. Mercury: Instrument technique - Cold vapor technique; wavelength - 253.7 nm; slit width - 0. 5 mm; lamp current - 3.0 mA; carrier gas and flow rate - argon, 1.1 L/min; sample flow rate - 5ml/min. Arsenic: Instrument technique - Flame vapor technique; wavelength - 193.7 nm; slit width - 0. 5 mm; lamp current - 6.0 mA; carrier gas and flow rate - acetylene, argon, 1.1 L/min; sample flow rate - 5ml/min. The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

2.8 Analysis of aflatoxins [3,9]

The procedure was followed for the analysis of aflatoxins B1, B2, G1 and G2 as per Official Analytical Methods of the American Spice Trade Association (ASTA, 1997).

Instrument details and operating parameters

Thermo Fisher High Performance Liquid Chromatography (HPLC) was used for the aflatoxins analysis. Column - Ultra C18, 250 X 4.6 mm, 5 µm particles; mobile phase - water: acetonitrile: methanol (65: 22.5: 22.5); flow rate - 1 ml/min; temperature - 35ºC; detector - fluorescence detector at 360 nm; injection - 20µl (Aflatoxins mixture and sample).

2.9 Analysis of pesticide residue[3]

The procedure followed for the analysis of pesticide residues was as per AOAC 2005. Pesticide residues were analysed by Gas Chromatography-Mass Spectra (GC-MS) (Instrument-Agilent, Detector-mass selective detector, Column specification-DB5MS, Carrier gas- Helium, Flow rate-1ml/min, Column length-30 m, Internal diameter-0.25 mm, Column thickness-0.25µm).

3.RESULT AND DISCUSSION

3.1 Macroscopical evaluation.

The colour, odour, taste and texture of Habb-e-Yarqaan were reported in table 2**.**

Table 2: Macroscopical evaluation of Habb-e-Yarqaan

S. No	Parameter	Batches		
		I	II	III
1.	Colour	Brown	Brown	Brown
2.	Odour	Characteristic	Characteristic	Characteristic
3.	Taste	Bitter	Bitter	Bitter
4.	Texture	Rough	Rough	Rough

Fig 1 Habb-E-Yarqan

Fig 2 Habb-E-Yarqan

3.2. Physicochemical analysis:

The total ash value, acid insoluble ash, alcohol soluble matter, water soluble matter, loss on drying, pH of 1% solution, pH of 10% solution of Habb-e-Yarqaan were reported in Table 3.

Table 3: Physiochemical Parameters Evaluation of Habb-e-Yarqaan

S. No	Parameters	Batches		
		I	II	III
1.	Total ash (%)	8.1476	8.3208	8.4961
2.	Acid insoluble ash	3.1852	3.2356	3.3615
3.	Alcohol sol. Matter	24.7642	24.5326	24.4681
4.	Water sol. Matter	46.7892	46.3251	46.8225
5.	Loss in wt. on drying at 105 ^{0}C	10.4087	10.4135	10.3942
6.	pH of 1% solution	4.01	4.02	4.01
7.	pH of 10% solution	3.96	3.98	3.97

3.3). Microbial contamination:

Total Bacterial load : $20x10^2$ (not more than 10^5/g)

Salmonella spp. : Nil

Escherichia coli : Nil

Total Fungal count : $15x10^2$ (not more than 10^3/g)

3.4). Aflatoxin contamination:

B1 : Nil (not more than 0.50 ppm)

B2 : Nil (not more than 0.10 ppm)

G1 : Nil (not more than 0.50 ppm)

G2 : Nil (not more than 0.10 ppm)

3.5). Heavy metal analysis:

The Lead, Cadmium, Arsenic, Mercury limit evaluation of **Habb-e-Yarqaan were reported in Table 4.**

Table 4: Heavy metal analysis of HABB-e-YARQAAN

Parameter analysed	Results			WHO Permissible limit
	B1	B2	B3	
Lead(Pb)	ND	ND	ND	10ppm
Cadmium(Cd)	ND	ND	ND	0.3ppm
Arsenic(As)	ND	ND	ND	3.0ppm
Mercury(Hg)	ND	ND	ND	1.0ppm

3.6). Pesticide residue:

The pesticide residue parameters of Habb-e-Yarqaan are reported in Table 5

Table 5: Pesticide residue Evaluation of Habb-e-Yarqaan

S. No	Test parameters	Units of Measurement	Results	Method of Testing
1	Aldrin	mg/kg	BLQ(LOQ-0.01)	AOAC 2007.01 by GC MSMS / LC MSMS
2	Chlordane (cis & trans)	mg/kg	BLQ(LOQ-0.01)	
3	Alachlor	mg/kg	BLQ(LOQ-0.01)	
4	Azinphos-methyl	mg/kg	BLQ(LOQ-0.01)	
5	Chlorfenviniphos	mg/kg	BLQ(LOQ-0.01)	
6	Endosulphan (all isomers)	mg/kg	BLQ(LOQ-0.01)	
7	Endrin	mg/kg	BLQ(LOQ-0.01)	
8	Chlorpyrifos	mg/kg	BLQ(LOQ-0.01)	
9	Chlorpyrifos-methyl	mg/kg	BLQ(LOQ-0.01)	
10	Cypermethrin	mg/kg	BLQ(LOQ-0.01)	
11	DDT	mg/kg	BLQ(LOQ-0.01)	
12	Deltamethrin	mg/kg	BLQ(LOQ-0.01)	
13	Diazinon	mg/kg	BLQ(LOQ-0.01)	
14	Dichlorvos	mg/kg	BLQ(LOQ-0.01)	
15	Ethion	mg/kg	BLQ(LOQ-0.01)	
16	Fenitrothion	mg/kg	BLQ(LOQ-0.01)	
17	Fenvalerate	mg/kg	BLQ(LOQ-0.01)	
18	Heptachlor	mg/kg	BLQ(LOQ-0.01)	
19	Hexachlorobenzene	mg/kg	BLQ(LOQ-0.01)	
20	Lindane(gamma-HCH)	mg/kg	BLQ(LOQ-0.01)	
21	Malathion	mg/kg	BLQ(LOQ-0.01)	
22	Parathion	mg/kg	BLQ(LOQ-0.01)	
23	Parathion methyl	mg/kg	BLQ(LOQ-0.01)	
24	Permethrin	mg/kg	BLQ(LOQ-0.01)	
25	Phosalone	mg/kg	BLQ(LOQ-0.01)	
26	Pirimiphos methyl	mg/kg	BLQ(LOQ-0.01)	

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

3.7). HPTLC Profile:

In the Present study of Habb-E-Yarqaan polyherbal formulation, different detecting method were tried to resolve the component of petroleum ether extract of Habb-E-Yarqaan. TLC of Petroleum ether extract was performed by using solvent system (Toluene: ethyl acetate - 9:1) and visualize under UV Chamber (366nm and 254nm), Iodine Chamber Visual region with anisaldehyde sulphuric acid. TLC of Petroleum ether extract of Habb-E-Yarqaan was shown in table 6.

Table 6: TLC solvent system for Petroleum ether extract of HABB-E-YARQAAN

Extract	Solvent System	Rf values			
		Under UV 366 nm (No of spot 9)	**Under UV 254nm (No of spot 7)**	**Iodine Vapours Chamber (No of spot 4)**	**Visible Region (Detecting Agent Anisaldehyde sulphuric acid) (No of spot 7)**
Petroleum Ether (40-60^0 C) Extract	Toluene: Ethyl acetate (9:1)	0.14 (Light Blue)	0.18(Black)	0.49 (Brown)	0.19 (Grey)
		0.19 (Brown)	0.36(Black)	0.53 (Grey)	0.49 (Dark Grey)
		0.22 (Yellow)	0.45(Black)	0.57 (Brown)	0.57 (Grey)
		0.28 (Blue)	0.53(Black)	0.90 (Brown)	0.63 (Grey)
		0.50(Red)	0.57(Black)	-	0.69 (Light Purple)
		0.56(Red)	0.81(Black)	-	0.90 (Purple)
		0.74 (Fluorescent Blue)	0.89(Black)	-	0.99 (Purple)
		0.79 (Yellow)	-	-	-
		0.93 (Blue)	-	-	-

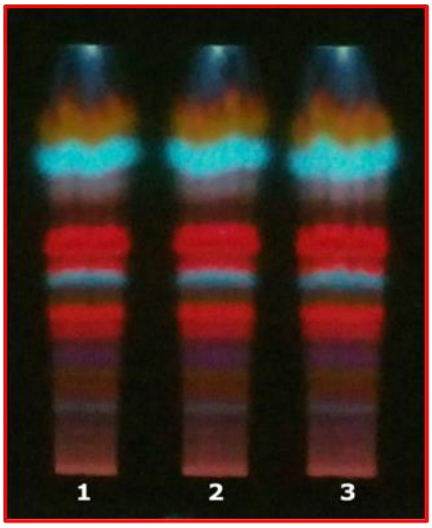

Fig. 3 At UV 366nm

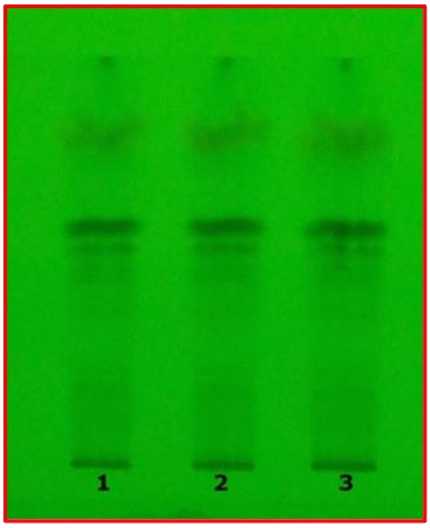

Fig. 4 At UV 254nm

Fig. 5 Expose to Iodine

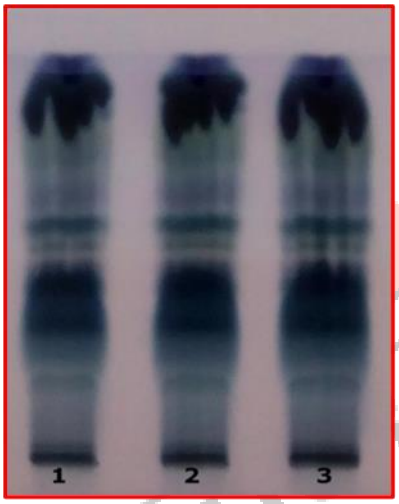

Fig. 6 After derivatization with Anisaldehyde sulphuric acid

Conclusion: Standards were established for polyherbal formulation for Habb-e-Yarqaan, which may be used as reference for preparation and standardization of the said formulation. In this work Standardization of Habb-e-Yarqaan with diverse ingredients including herbal and mineral origin drugs has been attempted with identification of its ingredients, formulation, physicochemical evaluation and TLC, which may help in preparing consistent and better efficacious formulations.

ACKNOWLEDGMENT

The authors would like to record their gratitude to Dr. N. Zaheer Ahmed, Director General, CCRUM, New Delhi for providing the necessary facilities and also the concern staff of our laboratory.

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