ijapc

E ISSN 2350 0204
www.ijapc.com

VOLUME 12 ISSUE 3

MAY 10, 2020

GREENTREE GROUP
PUBLISHERS

Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

Standardization and HPTLC Finger Printing Studies of Poly Herbal Formulation - Itrifal Haamaan

Pawan Kumar Sagar[1]*, R Murugeswaran[2], R.P. Meena[3], M.W.Ahmed[4], S.A.Ansari[5], S. Khair[6], and S A A Hashmi[7]

[1,2,4-7]Drug Standardization Research Institute, (Under CCRUM, Ministry of AYUSH., Govt. of India), PLIM Campus, II[nd] Floor, Kamla Nehru Nagar, Ghaziabad, U.P., India

[3]Central Council for Research in Unani Medicine,(Under Ministry of AYUSH., Govt. of India),61-62, Institutional Area, Janakpuri, New Delhi, India

ABSTRACT

Standardization is used to describe all measures under taken during the manufacturing process and quality control as well as quality assurance of drug leading to its reproducible quality. Most of the traditional and classical preparation of compound medicine are effective but still they lack in its standardization. Therefore, we need to develop standard techniques to standardize and validate herbal formulations. The drug Itrifal Haamaan is therapeutically used as Blood purifier, Anti-Vitiligo, Anti-Pityriasis, Prevents premature graying of hairs and Phlegmatic diseases. The drug Itrifal Haamaan was prepared in three different batches as per the guidelines of National Formulary of Unani Medicine(Part-IV). Present study is aimed to evaluate the pharmacopoeial standards using physico-chemical parameters; HPTLC fingerprinting, quality control and assurance parameters using WHO guidelines to ascertain the quality of drug. The physico-chemical data showed that the drug contain LOD/ Moisture (9.51%), Total Ash (1.65%), Acid in-soluble Ash(0.60%), Alcohol and Water soluble extractive matters (51.07%) & (71.04%), pH(1% solution) (4.77), pH(10% solution) (4.26), Bulk Density (1.5415 gm/ml),Reducing Sugar and Non-Reducing Sugar (58.54%) & (7.79%) and the TLC/HPTLC finger prints showed various spots at 254nm, 366nm and visible light (V-S reagent).The quality control study revealed the absence of microbial load, aflatoxins, heavy metals and pesticide residues, and can be considered safe for internal use and for curing for treated patients. The evaluated standards will be very useful for laying the phamacopoeial standards and as be supporting reference of Itrifal Haamaan and also in providing the quality medicine to needful human being.

Greentree Group Publishers

Received 13/12/20 Accepted 03/05/2020 Published 10/05/2020

KEYWORDS

Itrifal Haamaan, Physico-chemical standard, Quality Control and Assurance parameters, TLC/ HPTLC fingerprinting

Greentree Group Publishers

Received 13/12/20 Accepted 03/05/2020 Published 10/05/2020

INTRODUCTION

Standardization and validation of ASU herbal Drugs is not an easy challenge as various factors influence the bio efficacy and reproducible therapeutic effects. In order to obtain assured quality of herbal based products, care should be taken right from the proper identification of plants, season and area of collection of the drugs and their grading, drying, extraction, purification process and rationalizing the combination in case of poly-herbal drugs, Patel *et al*.,2006.

The subject of standardization and validation of herbal drugs is massively wide and deep. There are many seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function, Yadav *et al*.,2011. For the purpose of drug standardization research work of herbal formulated products, a complete profound knowledge is of utmost important.

Historically, herbal medicines have played a significant role in the management of both minor and major medical illness, Bahuguna *et al*.,2014. The quality assurance and quality control of herbal crude drugs and formulated products are important in justifying their acceptability in modern system of medicine. Hence it is required to conduct the research on drugs standardization to provide effective, curable and safe drugs to the needy mass suffering from various ailments.

The drug Itrifal Haamaan is one of the classical Unani poly-herbal compound formulations. The Unani drug standardization research studies of the Itrifal Haamaan is frequently recommended as a Blood purifier, for Vitiligo and Pityriasis debility. In most of the Asian, European and Arabian countries it is used since ancient times as traditional and alternative medicines. The drug has been used and Action wise reported as Daf-e-Bars (Anti-Vitiligo), Daf-e-Bahaq (Anti-Pityriasis) etc. Itrifal Haamaan is a dark brown semi-solid or mould form preparation with agreeable, aromatic odour, own smell and sweetish bitter in taste. Itrifal Haamaan was reported bioactive to contain phytochemical constituents such as Alkaloids, Flavonoides, Glycosides, Tannins, trace amount of volatile oil, Resins, Crude fibers etc. The preparation of the drug in different batches is based on traditional methods in accordance with the procedure given in NFUM,Part-IV ;Anonymous, 2006,(First Edition; Kabeeruddin Hkm. Mohammed. 2002;2006 and Anasri Arzani Keemiae M.A.2006.

In present research studies of ASU. Poly herbal drug Itrifal Haamaan have investigated and carried out, determinate of Loss on drying / Moisture contents % , Ash and Acid insoluble Ash contain % , pH values- 1% aqueous solution, 10 % aqueous solution, Extractives values, w/v - Alcohol soluble , Water soluble matter %, and Reducing Sugar, Non-Reducing Sugar, Bulk density, gm/ml using of physicochemical analysis techniques. Identifiably separated out of many phytochemical constituents spots by the help of HPTLC fingerprinting separation techniques examined preprepared TLC. Plates as stationary phase and applied in suitable mobile phase under treated in UV(254nm), UV(366nm) and dipped in 1% Vanillin - Sulphuric acid reagent examined under visible light region of separated spots of applied extract of drug samples mixture. Although Microbial load contamination was analysed as Total Bacterial Count, Total Fungal Count, *Escherichia coli, Salmonella typhai Spp., Staphylococcus aurous* microbial pathogenic bacteria's and Toxic, Hazards contamination, Heavy metals such as Lead(Pb),Cadmium(Cd), Mercury(Hg), and Arsenic (As) in ppm concentration, as well as Aflatoxins B1, Aflatoxins B2, Aflatoxine G1, Aflatoxine G2 in ppm concentration and estimation of 26 various required Pesticide Residues in mg/kg values were estimated and analysed as per compiled through WHO./API./UPI. Standard permissible limits basis, In the concerned of Quality Assurance and Quality Control as well as Pharmaco-vigilance, stability studies and advance research studies of formulated drug aspects of said investigated, analysed and estimated were essentially mandatory parameters for the purpose of evolution of safety and efficacy and drug standardization research of the drugs, Sagar *et al.*,2015.

MATERIAL AND METHODS

Ingredients used for preparation are given in Table a.

Drug preparation:

The formulated drug was prepared in different batches at Laboratory scale as per the ingredients compositions and guidelines of NFUM IV [13-14,16,19] classical text basis. The required quantities of all the ingredients were taken the pharmacopoeial quality. In these preparation of process take ingredients no. 1-17 and 19-22 were Cleaned, dried and powdered the said ingredient numbers sieved through mesh no.80 and kept separately. Ingredient no.18 was cleaned and its paste was prepared,

Heated the ingredients no. 23 was heated in a vessel on low flame until it boiled. The vessel was removed from the fire and immediately the paste of ingredient no. 18added along with Sodium Benzoate(0.1%) or Sodium methyl paraven(0.05%) and Sodium propyl paraven (0.02%) which are used as a antimicrobial, drug and food preservatives, powders of ingredient no. 1-17, 19-22 and mixed thoroughly to prepare a homogenous product. The preparation was allowed to cool to room temperature and stored in a tightly closed glass containerfree from moisture.

Table a The raw drug formulation is composed of the following mention ingredients:

S. No.	Unani Name	Botanical/ English Name	Part Used	Qty.	Reference
1.	Sheetraj	*Plumbago zeylancia* L.	Root	10 g	UPI, Part I, Vol. I, p.80
2.	Sazaj Hindi	*Cinnamomum tamala* Nees & Ebre.	Leaves	10 g	UPI, Part I,Vol. I, p. 78
3.	Mastagi	*Pistacia lentiscus*L.	Gum Resin	10 g	UPI, Part, Vol. V, p.50
4.	Anisoon	*Pimpinella anisum*L.	Fruit	10 g	UPI, Part I, Vol. II, p. 9
5.	Haasha	*Thymus serpyllum*L.	Leaves	10 g	Appendix
6.	Kundur	*Boswellia serrata* Roxb.	Resin	10 g	API, Part I, Vol. IV, p. 50
7.	Sad Kufi	*Cyperus rotundus*L.	Root	15 g	UPI, Part I, Vol. V, p.76
8.	Qust	*Saussurea hypoleuca* Spreng. Sy. *Aplotaxis auriculata* DC.	Root	15 g	UPI, Part I, Vol. I, 74
9.	Zanjabeel Khushk	*Zingiber officinale* Rosc.	Root	15 g	UPI, Part I,Vol. I, p. 88
10.	Zoofa Khushk	*Hyssopus officinalis*L.	Flower	15 g	UPI, Part I, Vol. II, p.97
11.	Filfil Siyah	*Piper nigrum*L.	fruit	20 g	UPI, Part I, Vol. IV, P. 38
12.	Filfil Daraz	*Piper longum*L.	Fruit	20 g	API, Part I, Vol. IV, p.105
13.	Narmushk	*Mesua ferrea*L.	flower	20 g	UPI, Part I, Vol. IV, p. 98
14.	Ghariqoon	*Agaricus alba*L.	Fruit	20 g	UPI, Part I, Vol. VI, p.27
15.	Ustukhuddus	*Lavandula stoechas*L.	Inflorescence	25 g	Appendix (APR 2012-13)
16.	Bisfayej	*Polypodium vulgare*L.	Rhizomes	25 g	UPI, Part I, Vol. II, p.29
17.	Post-e-Balela	*Terminalia bellirica* Roxb.	Pericarp	35 g	UPI, Part I, Vol. I, p. 17
18.	Aamla Munaqqa	*Emblica officinalis* Gaertn.	Fruit	35 g	UPI, Part I,Vol. I, p.5-6
19.	Aftimoon	*Cuscuta epithymum*L./ *Cuscuta reflexa* L.	Whole plant	35 g	UPI, Part I, Vol. III,p.1
20	Baobarang	*Embelia ribes* Burmf.	Fruit	35 g	UPI, Part I, Vol. I, p.19
21.	Turbud	*Operculina turpethum* (L) Silva Manso	Root	45 g	UPI, Part I, Vol. V, p. 105
22.	Post-e-Halela Kabuli	*Terminalia chebula* Retz.	Pericarp	70 g	UPI, Part I, Vol. I, p. 32
23.	Asal	Honey	As Such	1.7 Kg	UPI, Part II, Vol. I, p.82

Pharmacopoeial standards:

Pharmacopoeial research studies such as organoleptic characters, microscopical, macroscopical and physicochemical, TLC/HPLC., quality control and quality assurance parameters were carried out

1. Organoleptic Evaluation: Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc., using the sensory organs of our body. The organoleptic characters of the drugs samples were carried out based on the method described by Siddiqui *et al* [23].

2. Physico-chemical analysis: If the Water content of the drug is high it will easily deteriorate due to fungus, The Ash content indicates the total amount of inorganic material after complete incineration and the Acid insoluble ash is indicative of silicate impuritiespresent due to improper washing of the drug. The Alcohol and Water soluble extractives indicate the amount of active chemical constituents in a given amount of particular drug when extracted with respective solvents. Some of the useful tools in standardization of ASU herbal products such as Moisture content of the powdered sample at 105ºC, Ash values, Acid insoluble ash, Solubility in water and alcohol, pH values and Bulk density etc., were studies as per standard methods [26].

3. TLC/HPTLC finger printing analysis: The drug samples (2gm) were soaked in chloroform and alcohol separately for 18 hours and refluxed for ten minutes on water bath and filtered through What man N0.1 filter paper. The filtrates were concentrates and made up to 10 ml in volumetric flask with respective solvents. The procedure followed for the analysis of TLC and HPTLC was as per the standard method of Sagar *et al*., Wagner and Biadi [1-2,20,22].

4. Quality assurance and quality control parameters: The usage of ASU, herbal products along with higher safety margins, WHO has taken necessary steps to ensure quality assurance and quality control parameters with the modern techniques and application of suitable standards, The microbial load and heavy metal parameters using the tested powder drug were carried out as per the WHO guidelines [20]. The heavy metals were analyzed by Atomic Absorption Spectroscopy [6-12,17-18]. Aflatoxins were estimated by Kobra cell techniques using Agilent HPLC instruments as per the method ASTA [27] and pesticide residues were analyzed using GC-MS Agilent instruments equipped with Mass selective detector as per the method AOAC [1-2,17-18].

RESULT AND DISCUSSION

Organoleptic character of the formulated drug indicates that the drug is dark brown

semi-solid, mould drugform with agreeable aromatic odour,own smell and sweetish bitter in taste. The physico-chemical analysis such as LOD/Moisture content obtained in the drug was 9.51 % shows the amount of moisture present in the drug. The alcohol and water soluble extractives (51.07 % and 71.04 %) respectively might be due to presence of polar organic bio active chemical constituents and indicate the presence of inorganic constituents total Ash (1.65 %) and Acid in-soluble ash (0.60 %) indicate the presence of inorganic and metals form of substances, pH (1 % solution) (4.77), pH (10 % solution) (4.26), Alcohol soluble extractive ,ASE (51.07 %) and Water soluble extractive, WSE (71.04 %), Bulk density of granules(1.5418),Reducing Sugar and Non-Reducing Sugar (58.53) & (7.79)analyzed parameters were revealed of pharmacopeial parameters of semi-solid, mould form drug shown in (Table-1) respectively.

Table 1 Physico-chemical parameters

Parameters Analyzed	**Batch Numbers**			
	I	**II**	**III**	**Average value**
Extractives, w/v				
Alcohol soluble matter	50.92%	51.02%	51.27%	
Water soluble matter	70.64%	71.13%	71.36%	51.07% 71.04%
Ash values, w/w				
Total ash	1.54%	1.69%	1.73%	1.454%
Acid insoluble ash	0.55%	0.62%	0.64%	0.60%
pH values				
1% Aqueous solution	4.73	4.78	4.80	4.77
10% Aqueous solution	4.23	4.32	4.25	4.26
LOD./ Moisture content,w/w	9.30%	9.83%	9.40%	9.51%
Reducing Sugar	58.57%	57.72%	59.32%	58.53%
Non-Reducing Sugar	7.73%	7.59%	8.06%	7.79%
Bulk density, gm/ml	1.5333	1.5456	1.5456	1.5415

Thin Layer Chromatography

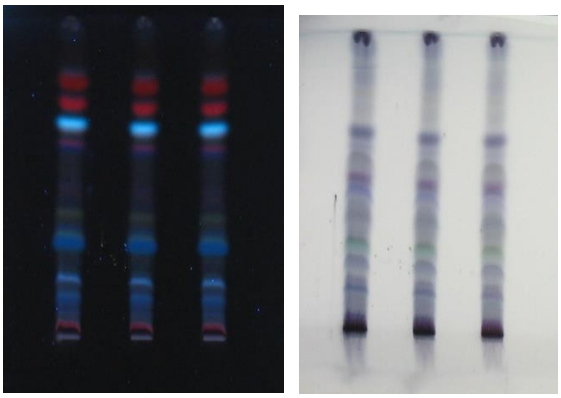
TLC/ HPTLC finger printing profiling of chloroform extract of 2g of sample done with 20 ml of chloroform separately and refluxed on water bath for 30min. The chloroform extracts were applied on TLC plate. Plate was developafter leaching out the sugar, 2g of drug with 40 ml of Chloroform and Ethyl alcohol was refluxed separately for 30 minutes and filtered. The filtrate up to 10 ml (approx.) concentrated on water bath and Chloroform extract applied on precoated aluminum TLC plate of silica gel 60 F_{254}using HPTLC automatic sample applicator. The plate was developed in Toluene - Ethyl acetate (8.0 : 2.0) solvent

system and allowed to dry in air and examined under UV (366nm). 10 major fluorescent spots at R_f 0.12, 0.17, 0.28 (blue), 0.30 and 0.36 (green), 0.56 (blue), 0.58 (pink), 0.63 (light blue), 0.69 & 0.77(red) were observed. Plate dipped in 1% Vanillin- Sulphuric *acid* reagent followed by heating at 105^{0}C for 5 minutes and examined under visible light.09 major spots at R_f 0.12, 0.20 (purple), 0.26 (green), 0.30 (purple), 0.34 (pinkish purple), 0.43 (violet), 0.46 (purple), 0.51 (green) & 0.61 (violet) were observed as shown in Figure-1 and Table-2, respectively.

Table 2 R_f values of Chloroform extract

Solvent system	***R*f Values**	
	366 nm	**Visible light-VS reagent**
Toluene : Ethyl acetate : (8.0 : 2.0)	0.12 (Blue)	0.12 (Purple)
	0.17 (Blue)	0.20 (Purple)
	0.28 (Blue)	0.26 (Green)
	0.30 (Green)	0.30 (Purple)
	0.36 (Green)	0.34 (Pinkish purple)
	0.56 (Blue)	0.43 (Violet)
	0.58 (Pink)	0.46 (Purple)
	0.63 (Blue light)	0.51 (Green)
	0.69 (Red)	0.61 (Violet)
	0.77 (Red)	

Ethanol extract applied on precoated aluminum TLC plate of silica gel 60 F_{254}using HPTLC automatic sample applicator. Plate was developed in Toluene - Ethyl acetate (8.0 : 2.0) solvent system, allowed to dry in air and examined under UV (366nm). 08 major fluorescent spots at R_f 0.12 (blue), 0.18 (light blue), 0.28 (blue), 0.35 (olive green), 0.56 (pink), 0.62 (light blue), 0.68 & 0.75 (red). Under UV (254nm) were observed. 06 major spots at R_f 0.47, 0.54, 0.61, 0.64, 0.67 & 0.71 (green). The plate dipped in 1% Vanillin - Sulphuricacid reagent followed by heating at 105^{0}C for 5 minutes and examined under visible light. 07 major spots at R_f 0.20, 0.23 (purple), 0.29(olive green), 0.38(pinkish purple), 0.47(pink), 0.53(olive green) & 0.63 (pinkish purple) were observed as shown in Figure - 2 and Table-3 respectively.



B-I B-II B-III **B-I B-II B-III**
UV 366nm **Visible Light (After derivatization of V-S Reagent)**

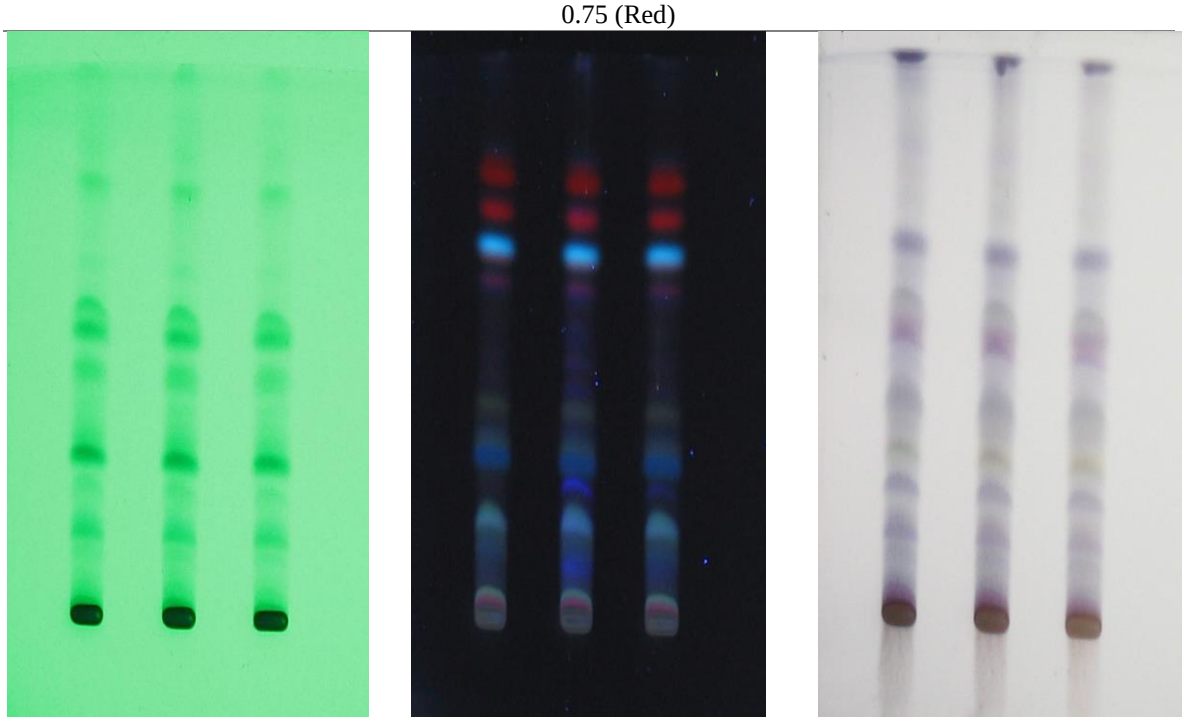
Solvent System: Toluene : Ethyl acetate (8.0 : 2.0)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III

Figure 1 TLC/HPTLC Photo of Chloroform Extract

Table-3 R_f values of Alcohol extract

Solvent system	R_f Values		
	254 nm	**366 nm**	**Visible light-VS reagent**
	0.47 (Green)	0.12 (Blue)	0.20 (Purple)
	0.54 (Green)	0.18 (Light Blue)	0.23 (Purple)

Toluene : Ethyl acetate (8.0 : 2.0)	0.61 (Green)	0.28 (Blue)	0.29 (Olive green)
	0.64 (Green)	0.35 (Olive green)	0.38 (Pinkish purple)
	0.64 (Green)	0.56 (Pink)	0.47 (Pink)
	0.67 (Green)	0.62 (Light Blue)	0.53 (Olive green)
	0.71 (Green)	0.68 (Red)	0.63 (Pinkish purple)
		0.75 (Red)	



B-I B-II B-III **B-I B-II B-III** **B-I B-II B-III**

UV 254 nm **UV366 nm** **V-S Reagent**

Solvent System: Toluene : Ethyl acetate (8.0 : 2.0)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III

Figure 2 TLC/HPTLC Photo of Alcohol Extract

Quality Assurance and Quality Control Parameters:

The analysis of microbial load (Table 4) present in the drug showed that the total bacterial count (TBC) and total fungal count(TFC) was revealed 600 and 500 cfu/gm.The detection of themicrobial load was under the permissible limits of WHO guideline.

Table 4 Analysis of Microbial load

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Total Bacterial Count	700 cfu/gm	10^5 cfu/gm
2	Total Fungal Count	100 cfu/gm	10^3 cfu/gm
3	*Escherichia coli*	Absent	Absent
4	*Salmonella typhai Spp.*	Absent	Absent
5	*Staphylococcus aurous*	Absent	Absent

Finally, the results obtained by heavy metal analyses, Aflatoxins and pesticide are given in Table 5, 6 and 7.

Table 5 Estimation of Heavy Metals

S.No.	Parameter Analyzed	Results	WHO Limit
1	Lead	Not detected	10 ppm
2	Cadmium	Not detected	0.3 ppm
3	Mercury	Not detected	1.0 ppm
4	Arsenic	Not detected	3.0 ppm

Table 6 Estimation of Aflatoxins

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Aflatoxins B1	Not detected	0.5 ppm
2	Aflatoxins B2	Not detected	0.1 ppm
3	Aflatoxine G1	Not detected	0.5 ppm
4	Aflatoxine G2	Not detected	0.1 ppm

Table 7 Estimation of Pesticide Residues

S.N0.	Parameter Analyzed	Results	WHO Limit (mg/kg)
1	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	Not detected	1.0
2	HCH (sum of all isomers)	Not detected	0.3
3	Endosulphan (all isomers)	Not detected	3.0
4	Azinphos-methyl	Not detected	1.0
5	Alachlor	Not detected	0.02
6	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	Not detected	0.05
7	Chlordane (cis& tans)	Not detected	0.05
8	Chlorfenvinphos	Not detected	0.5
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Not detected	0.05
10	Endrin	Not detected	0.05
11	Ethion	Not detected	2.0
12	Chlorpyrifos	Not detected	0.2
13	Chlorpyrifos-methyl	Not detected	0.1
14	Parathion methyl	Not detected	0.2
15	Malathion	Not detected	1.0
16	Parathion	Not detected	0.5
17	Diazinon	Not detected	0.5
18	Dichlorvos	Not detected	1.0
19	Methidathion	Not detected	0.2
20	Phosalone	Not detected	0.1
21	Fenvalerate	Not detected	1.5
22	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	Not detected	1.0
23	Fenitrothion	Not detected	0.5
24	Deltamethrin	Not detected	0.5
25	Permethrin (sum of isomers)	Not detected	1.0
26	Pirimiphos methyl	Not detected	4,0

CONCLUSION

In the present investigated research study various standardization parameters such as physico-chemical, TLC / HPTLC finger print and WHO parameters were revealed and carried out can be laid down as reference standards for the analysed compound drug. From the present studies it can be concluded that the formulated Itrifal Haamaanis safe, effective, free from any toxic, hazardous substance it is an economic drugand the efficacy of the drug can be effectively used in traditional alternative medicine as a Blood purifier,Anti-Vitiligo, Anti-Pityriasis,Prevents premature graying of

hairs and Phlegmatic diseases. Referential information have also been evaluated by conducting the clinical studies on patients suffering of Vitiligoand PityriasisPrevents premature graying of hairs and Phlegmatic diseases andcan be considered incorporation of pharmacopoeial monograph.

ACKNOWLEDGMANT

The authors are extremely thankful to the Director General, CCRUM, New Delhi, under Ministry of AYUSH., Govt. of India for his valuable guidance, encouragement and for providing necessary research facilities to carry out the research studies as well as also thankful to all dedicated research staff of the research Institute for providing full cooperation and support to complete this research work.

REFERENCES

1. Sagar, P.K., Murugeswaran, R., Meena, R., Mageswari, S., and Sri, P.Meera Devi, Khair, S. (2020). Standardization and HPTLC. Fingerprinting study of Poly Herbal Unani Formulation-Habb-e-Sara Khas. International Journal of Traditional and Complementary Medicine,5(21),1-13.
2. Sagar, P.K., Kazmi, M.H., Siddiqui, J.I., and N. Rasheed, M.A. (2015). Pharmacopeial Standard development ,HPTLC, Fingerprinting and Physicochemical Research Studies of Unani Anti-Paralytic drug Majoon-e-Seer Alwi Khani. European Journal of Bio Pharmaceutical Science ,2(5),402-411.
3. Bahuguna, Y., Zaidi, S., Kumar, N., and Rawat, K.(2014).Standardization of Polyherbal Marketed Formulation TriphalaChurna, Research and Review. Journal of Pharmacognocy and Phytochemistry,2(3),28-35.
4. Anonymous.(2011). National Formulary of Unani Medicine VI . Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi.
5. Yadav, P., Mahour, Y., and Kumar, A.(2011). Standardization and Evaluation of Herbal Drug Formulations. Journal of Advance LaboratoryResearch in Biology,2(4),161-166.
6. Anonymous.(2009). The Unani Pharmacopeia of India I (Vol. 6). published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi.
7. Anonymous.(2008).The Ayurvedic Pharmacopeia of India I (Vol. 6). published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi.
8. Anonymous.(2008). The Unani Pharmacopeia of India I (Vol. 5). published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi,80-81.
9. Anonymous.(2007).The Unani Pharmacopoeia of India I (Vol. 1). Govt. of India, Min. of Health & Family Welfare, New Delhi.
10. Anonymous.(2007). The Unani Pharmacopoeia of India I(Vol. 3). Govt. of India, Min. of Health & Family Welfare, New Delhi.
11. Anonymous.(2007). The Unani Pharmacopeia of India I (Vol. 4). Govt. of India, Min. of Health & Family Welfare, New Delhi.
12. Anonymous.(2007).The Unani Pharmacopoeia of India I (Vol. 2). Govt. of India, Min. of Health & Family Welfare, New Delhi,51-52,81-82, 89-90 and 91-92.
13. Anonymous.(2006). National Formulary of Unani Medicine IV (First

Edition), Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi.

14. Kabeeruddin, Hakim M. (2006). Al-Qarabadeen, Central Council for Research in Unani Medicine, Janakpuri, New Delhi.

15. Patel, P.M., Patel, N.M., Goyal, R.K.(2006).Quality control of herbal products, The Indian Phamacist,5(45),26-30.

16. Anasri Arzani Keemiae, M.A.(2006).Urdu translation of Qarabadeene Qadri by Noor Kareem, H. M., C.C.R.U.M., New Delhi.

17. Anonymous.(2005). Official Methods of Analysis (AOAC). International Horwitz, W., Latimer, G. W. 18th Edn. AOAC International: Maryland, chapter 3,10-11,chapter 10,18-23.

18. Anonymous.(2005).Official Methods of Analysis(AOAC), International Horwitz W., Latimer, G. W.18thEdn. AOAC International;chapter 26,17.

19. Kabeeruddin, Hakim M.(2002). Bayaz-e-Khas al maruf ilaj-ul-amraz,Aijaz. Publishing House, New Delhi.

20. Anonymous.(1998).Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva.

21. Anonymous.(1997).Official Analytical Methods of the American Spice Trade Association (ASTA), Inc. 4th Edn. New Jersey.

22. Wagner, H. and Biadi, S. (1996). Plant Drug Analysis - A Thin Layer Chromatography Atlas, Springer-Verlag, 2ndEdn., Germany.

23. Siddqui, Hakim M. A. (1995).Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs 24-25th January (Appendix). Central Council for Research in Unnai Medicine, New Delhi.

24. Anonymous.(1991). Physico-chemical standards of Unani Formulations II C.C.R.U.M., Ministry of Health and Family Welfare, New Delhi.

25. Anonymous.(1990). The Ayurvedic Pharmacopoeia of India I(Vol.1). Govt. of India, Min. of Health & Family Welfare, New Delhi.

26. Anonymous.(1987). Physico-chemical standards of Unani Formulations II C.C.R.U.M., Ministry of Health and Family Welfare, New Delhi.

27. Anonymous.(1986). Physico-chemical standards of Unani Formulations II C.C.R.U.M., Ministry of Health and Family Welfare, New Delhi.