EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 12
210-218
Year: 2020

DRUG STANDARDIZATION AND MONOGRAPHS DEVELOPMENT, HPTLC. FINGER PRINTING RESEARCH STUDIES OF POLYHERBAL FORMULATION - HABB-E-NISHAT JADEED

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Article Received on 26/09/2020 **Article Revised on 16/10/2020** **Article Accepted on 06/11/2020**

ABSTRACT
Standardization is used to describe all measures under taken during the manufacturing process and quality control of drug leading to its reproducible quality. Most of the traditional medicine are effective but still they lack in its standardization. Therefore we need to develop standard techniques to standardize and validate of the herbal formulations. The drug Habb-e-Nishat Jadeed is therapeutically useful in the treatment of Zof-e-Bah (Sex debility). The drug Habb-e-Nishat Jadeed was prepared in three different batches as per the guidelines of National Formulary of Unani Medicine(Part-VI). Present study is aimed to evaluate the pharmacopoeial standards using physico-chemical parameters; HPTLC fingerprinting, quality control and assurance parameters using WHO guideline to ascertain the quality of drug. The physico-chemical data showed that the drug contain LOD/moisture (2.33%), total ash (7.7%), acid in-soluble (4.32%), alcohol and water soluble extractive matter (20.39%) & (23.10%), pH (1% solution), (5.59), pH (10% solution) (5.66), ASS Extractive (16.13%) and CSS Extractive (7.86%), Bulk density of granules(0.5467) 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 metal and pesticide residues. The evaluated standards will be very useful for laying the pharmacopoeial standards of Habb-e-Nishat Jadeed and also in providing the quality medicine to needful human being.

KEYWORDS: Habb-e-Nishat Jadeed, Physico-chemical quality, Quality Control and Assurance parameters, TLC/ HPTLC fingerprinting and Unani Compound drug.

INTRODUCTION
Standardization 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 and based herbal products, care through pharmaco-vigilance and care should be taken right from the proper identification of plants, season and area of collection, grading, drying, extraction, purification process and rationalizing the combination in the case of poly-herbal drugs (Patel *et al.*, 2006) and and (Sagar *et al.*, 2020).

The subject of standardization 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 (Sagar *et al*., 2020).

The drug Habb-e-Nishat Jadeed (Anonymous, 2006) is one the classical Unani poly-herbal compound formulations. It is therapeutically useful in the ailments of Zof-e-Bah (Sexual debility). The Unani drug standardization research studies of the drug Habb-e-Nishat Jadeed is frequently recommended as an Anti-Sex 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 Moharrik (Stimulant) and therapeutically used in Zof-e-Bah (Sex debility) etc. Habb-e-Nishat Jadeed is a Brown colour silver coated, pills form preparation with agreeable, aromatic odour and pungent taste. Habb-e-Nishat Jadeed was reported bioactive to contain phytochemical constituents such as Alkaloids, Glycosides, Tannins, Crude fibres etc. The preparation of the drug in different batches is based on traditional methods in accordance with the procedure given in NFUM, Part-VI (Anonymous, 2011).

MATERIAL AND METHODS

Ingredients used for preparation

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

1.	Sat-e-Salajeet	*Euphorbia royleana* Boiss and *Trifolium repens* L.	Silajit liquid Extract	30g
2.	Kushta-e-Musallas	--	Unani Mineral drug	10g
3.	Kushta-e-Nuqra	--	Unani Mineral drug	10g
4.	Kushta-e-Yaqut	--	Unani Mineral drug	10g
5.	Zafran Khalis	*Crocus sativus* Linn.	Dried stigma and style	30g
6.	Araq-e-Gulab	*Rosa damascena* Mill.	Water distillate	50ml
7.	Jauzbuwa, UPI-I	*Myristica fragrans* Houtt.	Endosperm	50 g
8.	Jawitri, UPI-VI	*Myristica fragrans* Houtt.	Arillus	50g
9.	Qaranfal, UPI-I	*Syzygium aromaticum* (L.)Merr & L.M. Perry	Flower bud	50g
10.	Samundar Sokh	Argyreia nervosa (Burm.f.) Boj.	Seed	50g
11.	Aaqarqarha, UPI-II	*Anacyclus pyrethrum* D.C.	Root	50g
12.	Sumbul-ut-Teeb, UPI-I	*Nardostachys jatamansi* DC.	Rhizome	50g
13.	Sad Kufi, UPI-V	*Cyperus rotundus* Linn.	Rhizome	50g
14.	Darchini, UPI-I	*Cimamomum zeylanicum* Blume.	Inner stem bark	50g
15.	Dar-e-Filfil, API-IV	*Piper longum* Linn.	Fruit	50g
16.	Khulanjan UPI-II	*Alpenia galanga* (L.) SW.,	Rhizome	50g
17.	Tukhm-e-Utangan, API-IV	*Bleferis persica* (Burm. f) O Kuntze.	Seed	50g
18.	KuchlaMudabbar, API-IV	*Strychnos nux-vomica* Linn.	Seed	25g
19.	Beer Bahuti	Velvety Tick, Cochineal Insect	(Animal)	2.5g
20.	Kharateen	Pheritima spp. (Earthworm)	(Animal)	2.5g
21.	Mahi Rubian	Palemoncurtinus (Shrimps)	(Animal)	10g
22.	Shingraf	Compound of Mercury and Sulphur	(Mineral)	1g.
23.	Araq-e-Heel	*Elettaria cardamomum* Maton.	Water distillate	50ml
24.	Barg-e-Pan Sabz	*Piper betle* Linn.	Water extract	200g
25.	Mastagi Roomi, UPI-V	*Pistacia lenticus* Linn.	Resin	3g
26.	Warq-e-Nuqra	Silver foils /leaves	--	Q.S.

Drug preparation

The formulated drug was prepared in different batches at Laboratory scale as per the ingredients compositions and guidelines of NFUM-VI(Anonymous, 2011). The required quantities of all the ingredients were taken of pharmacopoeial quality. Take all the ingredients of pharmacopoeial quality. Clean, dry and powder the ingredient number 7 to 17, sieve through 100 mesh and keep separately. Take the required quantity of ingredient number 1 to 4, powder it using mortar and pestle and keep separately. Take the required quantity of ingredient number 5, grind using mortar and pestle with Arq-e-Gulab till it becomes smooth paste. Prepare Arq (Distillate) of ingredient number 6 (Gulab), 23 (Heel) and keep separately. Detoxify* the ingredient number 18 and make its powder and sieve through 80 mesh and keep separately. Clean, dry and powder the ingredient number 19 to 21 of animal origin drugs, sieve through 80 mesh and keep separately. Powder of ingredient number 22 using Tamera Kharal, 25 by mortar and pestle, sieve through 100 mesh and keep separately. Take the required quantity of ingredient number 24 (fresh pan leaves), clean, dry, cut into small pieces, immerse in boiling water, leave for few minutes, collect the filtrate and keep the filtrate separately. Take the powder of ingredient number 1 to 5, 7 to 22 and 25, mix them thoroughly and

keep separately. Add Arq (distillate) of ingredient number 6 and 23 to the mixed powders and stire thoroughly. Add the filtrate of ingredient number 24 to the above mixed powders to prepare the lubdi mass. Roll the lubdi mass, convert into sticks of required size, thickness and cut into pieces using a knife. Round the cut pieces to give the shape of Huboob of required size and weight. Roll the Hubbob over the ingredient number 26 (Warq-e-Nuqra – Silver foils / leaves). Dry the Huboob under shade and store in a tightly closed glass container.

Note: - * Detoxification of Kuchla - The Kuchla seeds were soaked in water for first 5 days with change of water daily next 2 days in Cow's milk with change of milk daily, washed with water and were boiled in Cow's milk till the seed coat becomes soft. The seed coat and embryo part were removed and used in preparation.
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 Siddique *et al*. (1995) and Sagar *et al*.(2020).

2. **Physico-chemical analysis:** If the water content is high the drug can easily be deteriorated due to fungus, The ash content indicates the total amount of inorganic material after complete incineration and the acid insoluble ash is an indicative of silicate impurities might be due to improper washing of the drug. The alcohol and water soluble extractive indicates the amount of active chemical constituents in a given amount of particular drug when extracted with respective solvent. 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., are useful tools were studies as per standard methods(Anonymous,1987) and Sagar *et al*.(2020).

3. **TLC/HPTLC finger printing analysis:** The drug samples (2gm) were soaked in chloroform and alcohol separately for 18 hours and refluxed for 30 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 (Saxena and Yadav, 1983). The procedure followed for the analysis of TLC and HPTLC was as per the standard method of (Wagner and Blad, 1996) and Sagar *et al*.(2020).

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 (Anonymous,1998). The heavy metals were analyzed by Atomic Absorption Spectroscopy(Anonymous, 2005); aflatoxins were estimated by Kobra cell techniques using Agilent HPLC instruments as per the method ASTA (Anonymous, 1997) and pesticide residues were analyzed using GC-MS Agilent instruments equipped with Mass selective detector as per the method AOAC (Anonymous, 2005) and Sagar *et al*.(2020).

RESULT AND DISCUSSION

Organoleptic character of the formulated drug indicates that the drug is dark brown colour, aromatic odour and pungent taste. The physico-chemical analysis such as LOD/Moisture contain obtained in the drug was 2.33% shows the amount of moisture content present in the drug. The alcohol and water soluble extractive (20.39%) and (23.10%) might be due to the extractions the presence of polar organic bio active chemical constituents and indicate the presence of inorganic constituents, total ash (7.7%) and acid in-soluble ash (4.32%) indicate the presence of inorganic matter and metals form of substance, pH (1% solution) (5.59), pH (10% solution) (5.66), Alcohol soluble successive extractive, ASSE (16.13%) and Chloroform soluble successive extractive, CSSE(7.86%), Bulk density of granules (0.5467) analysed parameters were revealed of pharmacopeial parameters of pills form drug shown in (Table-1) respectively.

Thin Layer Chromatography

TLC/ HPTLC finger printing profiling of chloroform extract of 2g of sample with 20ml of chloroform separately and reflux on water bath for 30min. Filter and concentrate to 5ml and carry out the thin layer chromatography, Extract 2g of sample with 20ml of chloroform and alcohol separately and reflux on water bath for 30min. Filter and concentrate to 5ml and carry out the thin layer chromatography. Apply the chloroform extracts on TLC plate. Develop the plate using Toluene: Ethyl acetate: Formic acid (8.5: 1.5: 0.2) as mobile phase. After development allow the plate to dry in air and it was scanned and examine under UV (254nm), it shows 12 major spots at R_f 0.85, 0.78, 0.66 (Dark green), 0.60 (Green), 0.58, 0.42, 0.38 (Dark green), 0.32, 0.28, 0.23 (Green), 0.20 and 0.11 (Dark green). same it was scanned and examine under UV (366nm), it shows 21 major spots at R_f 0.95 (Blue), 0.81 (Violet), 0.75 (Blue), 0.69 (Violet), 0.65 (Red), 0.61 (Brown), 0.59 (Fluorescent blue), 0.54 (Red), 0.51, 0.48 (Grey), 0.46 (Brown), 0.39 (Grey), 0.32 (Violet), 0.28

(Pink), 0.24 (Grey), 0.21 (Violet), 0.19, 0.17, 0.15 (Red), 0.11 (Grey), 0.09 (Violet) and 0.07 (Red). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110° about 5 min observed same it was scanned and examine under visible light, the plate shows 17 major spots at R_f 0.84 (Grey), 0.75 (Brown), 0.71 (Grey), 0.66 (Yellow), 0.64 (Grey), 0.55 (dark grey), 0.51, 0.45 (Grey), 0.43 (Pink), 0.41 (Yellow), 0.38 (Grey), 0.30 (Violet), 0.27 (Grey), 0.21 (Green), 0.19, 0.12 (Grey) and 0.09 (Pink),shown in (Fig.-1,2,3 and Table-2) respectively .

Same applied the alcohol extracts on TLC plate. Develop the plate using Toluene : Ethyl acetate (9 : 1) as mobile phase. After developments allow the plate to dry in air and it was scanned and examine under UV (254nm), it shows 15 major spots at R_f 0.95 (Dark green), 0.81 (Green), 0.78, 0.67 (Dark green), 0.64, 0.58, 0.51 (Green), 0.48 (Dark green), 0.42, 0.37 (Green), 0.32, 0.28 (Dark green), 0.22, 0.18 (Green) and 0.08 (Dark green). same it was scanned and examine under UV (366nm), it shows 8 major spots at R_f0.59, 0.55 (Violet), 0.48 (Fluorescent blue), 0.33 (Violet), 0.31 (Fluorescent green), 0.12, 0.08 (Violet) and 0.05 (Grey). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110° about 5 min ,observe same it was scanned and examine under visible light, the plate shows 17 major spots at R_f 0.88 (Grey), 0.85 (Yellow), 0.78 (Violet), 0.73 (Yellow), 0.66 (Violet), 0.61 (Yellow), 0.55 (Grey), 0.49 (Violet), 0.45 (Pink), 0.42, 0.38 (Dark grey), 0.36 (Violet), 0.31 (Dark grey), 0.23 (Grey), 0.16 (Violet), 0.12 (Dark grey) and 0.08 (Light grey). shown in (Fig.-4,5,6 and Table-3) respectively.

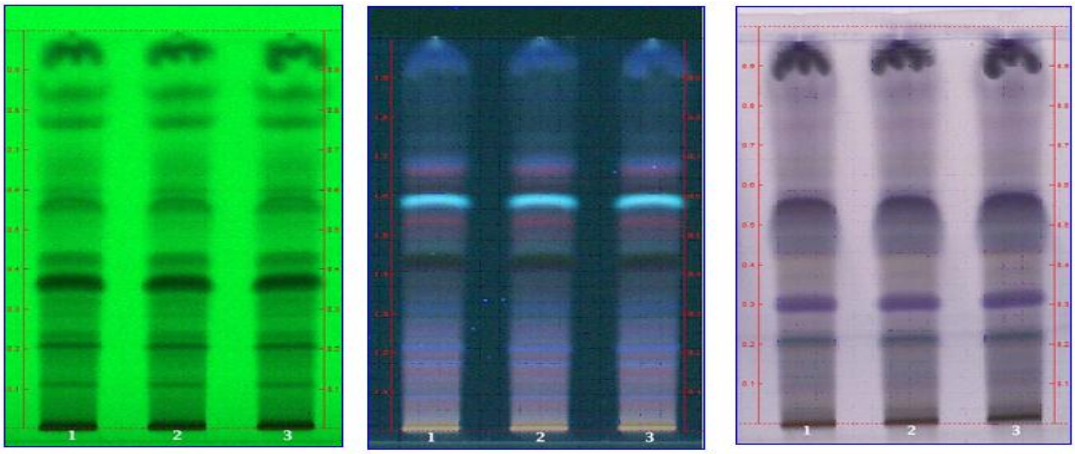
Table-1: Physico-chemical parameters.

Parameters Analyzed	Batch Numbers		
	I	II	III
Extractives, w/v Alcohol soluble matter Water soluble matter	 20.38% 23.09%	 20.41% 23.12%	 20.38% 23.09%
Ash values, w/w Total ash Acid insoluble ash	 7.70% 4.32%	 7.70% 4.32%	 7.70% 4.32%
pH values 1% Aqueous solution 10% Aqueous solution	 5.58 5.66	 5.60 5.67	 5.60 5.67
LOD./ Moisture content,w/w	2.32%	2.34%	2.33%
Alcohol soluble extractive values (Soxhlet-Hot extraction),w/v	16.81%	16.80%	14.80%
Chloroform soluble extractive values (Soxhlet-Hot extraction),w/v	7.85%	7.86%	7.87%
Bulk density, gm/ml	0.5492	0.5413	0.5498

Table-2:R_f values of chloroform extract.

Solvent system	R_f Values		
	254nm	366nm	VS reagent
Toluene : Ethyl acetate : Formic acid (8.5 : 1.5 : 0.2)	0.85(Dark green)	0.95(Blue)	0.84(Grey)
	0.75(Dark green)	0.81(Violet)	0.75(Brown)
	0.66(Dark green)	0.75(Blue)	0.71(Grey)
	0.60(Green)	0.69(Violet)	0.66(Yellow)
	0.58(Dark green)	0.65(Red)	0.64(Grey)
	0.42(Dark green)	0.61(Brown)	0.55(Dark grey)
	0.38(Dark green)	0.59(Fluorescent blue)	0.51(Grey)
	0.32(Green)	0.54(Red)	0.45(Grey)
	0.28(Green)	0.51(Grey)	0.43(Pink)
	0.23(Green)	0.48(Grey)	0.41(Yellow)
	0.20(Dark green)	0.46(Brown)	0.38(Grey)
	0.11(Dark green)	0.39(Grey)	0.30(Violet)
		0.32(Violet)	0.27(Grey)
		0.28(Pink)	0.21(Green)
		0.24(Grey)	0.19(Green)
		0.21(Violet)	0.12(Grey)
		0.19(Red)	0.09(Pink)
		0.17(Red)	

		0.15(Red)	
		0.11(Grey)	
		0.09(Violet)	
		0.007(Red)	



UV - 254nm **UV - 366nm** **V - S Reagent**

Solvent System: Toluene: Ethyl acetate: Formic acid (8.5: 1.5 : 0.2)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III
Figure 1: TLC/HPTLC Photo of Chloroform Extract.

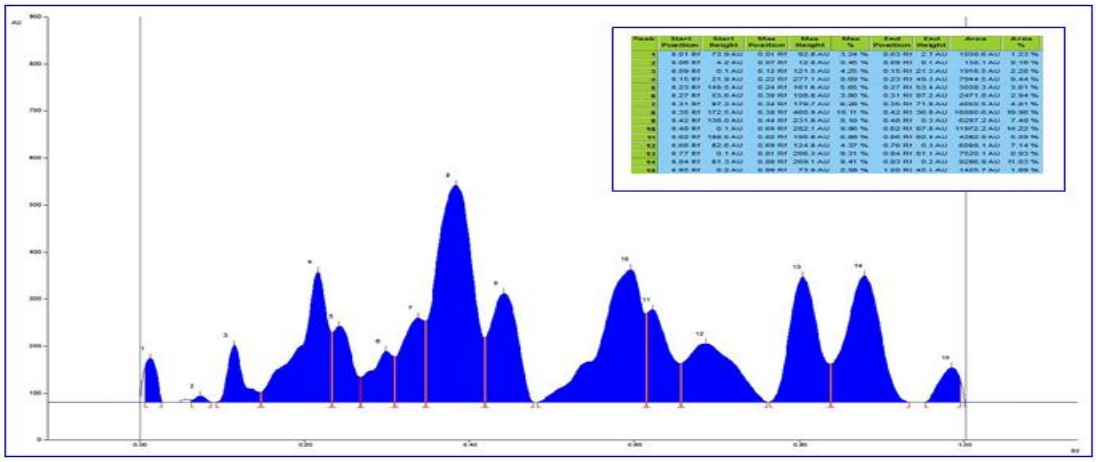


Figure-2: HPTLC finger print of Habb-e-Nishat Jadeed chloroform extract at 254nm.

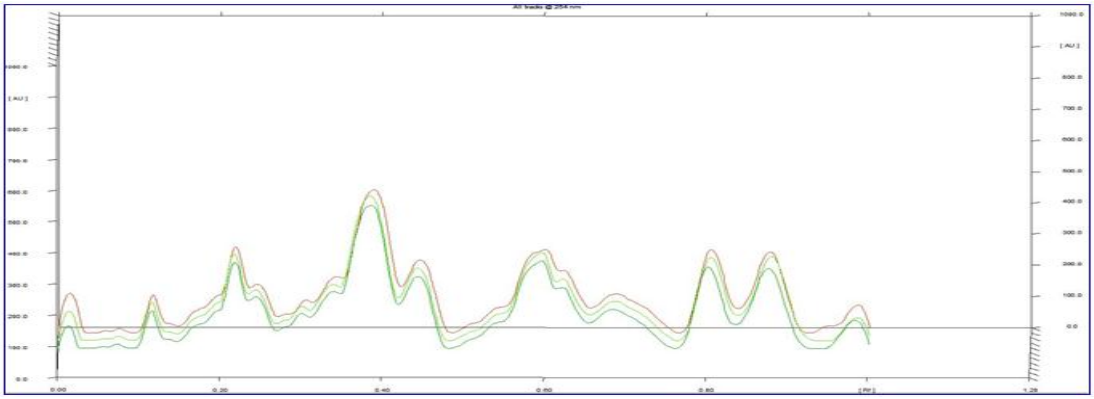
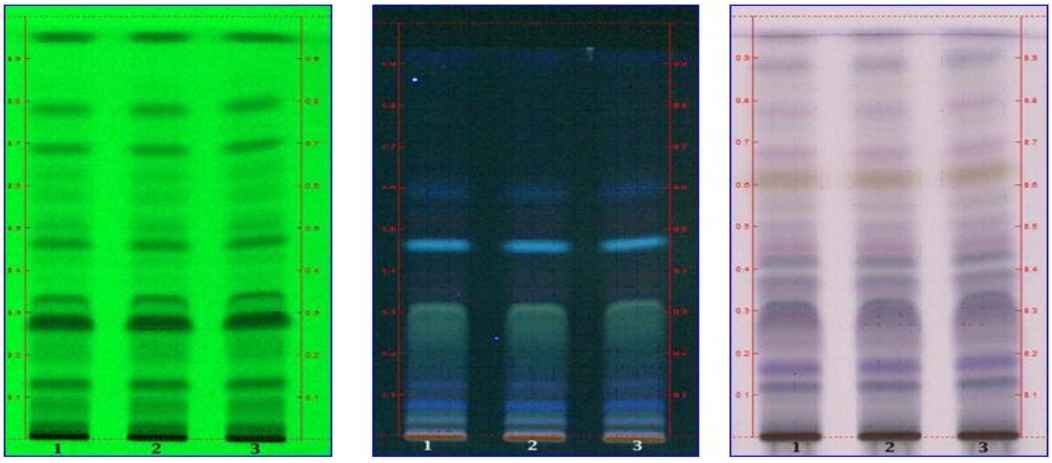


Figure-3: Densitometric chromatogram of Habb-e-Nishat Jadeed chloroform extracts at 254nm.

Table-3: R_f values of alcohol extract.

Solvent system	R_f Values		
	254nm	366nm	VS reagent
Toluene : Ethyl acetate (9 : 1)	0.95(Dark green)	0.59(Violet)	0.88(Grey)
	0.81Green)	0.55(Violet)	0.85(Yellow)
	0.78(Dark green)	0.48(Fluorescent blue)	0.78(Violet)
	0.67(Dark green)	0.33(Violet)	0.73(Yellow)
	0.64(Green)	0.31(Fluorescent green)	0.66(Violet)
	0.58(Green)	0.12(Violet)	0.61(Yellow)
	0.51(Green)	0.08(Violet)	0.55(Grey)
	0.48(Dark green)	0.31(Blue)	0.49(Violet)
	0.42(Green)	0.19(Blue)	0.45(Pink)
	0.37(Green)	0.12(Violet)	0.42(Dark grey)
	0.32(Dark green)	0.08(Violet)	0.38(Dark grey)
	0.28(Dark green)	0.05(Grey)	0.36(Violet)
	0.22(Green)		0.31(Dark grey)
	0.18(Green)		0.23(Grey)
	0.08(Dark green)		0.16(Violet)
			0.12(Dark grey)
			0.08(Light grey)



UV – 254nm **UV - 366nm** **V - S Reagent**

Solvent System: Toluene : Ethyl acetate (9 : 1)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III
Figure 4: TLC/HPTLC Photo of Alcohol Extract.

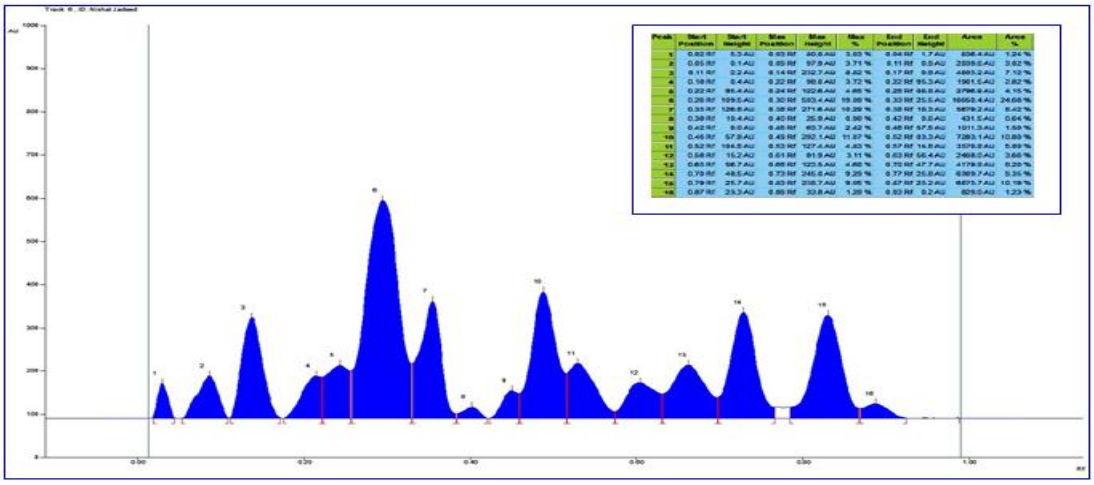


Figure-5: HPTLC finger print of Habb-e-Nishat Jadeed alcohol extract at 254nm.

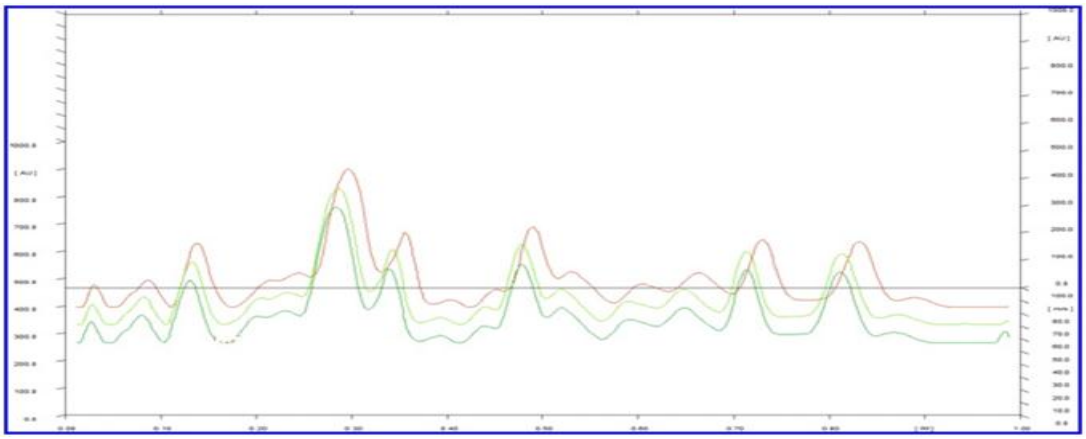


Figure-6: Densitometric chromatogram of Habb-e-Nishat Jadeed alcohol extracts at 254nm Quality Assurance and Quality Control Parameters.

The analysis of microbial load 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 the microbial 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	600 cfu/gm	10^5cfu/gm
2	Total Fungal Count	500 cfu/gm	10^3cfu/gm
3	*Escherichia coli*	Absent	Absent
4	*Salmonella typhai Spp.*	Absent	Absent
5	*Staphylococcus aurous*	Absent	Absent

Table-5: Estimation of Heavy Metals.

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Lead	3.50ppm	10ppm
2	Cadmium	0.04ppb	0.3ppm
3	Mercury	Not detected	1.0ppm
4	Arsenic	0.12 ppm	3.0ppm

Table-5: Estimation of Aflatoxins.

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

Table-6: 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 studies of various standardization parameters such as physico-chemical, TLC/HPTLC finger printing analysis and WHO parameters were revealed and carried out can be laid down as reference standards of the drug Habb-e-Nishat Jadeed. From the present studies it can be concluded that the formulated Habb-e-Nishat Jadeed is safe, effective and free from any toxic, hazardous substance. It is an economic drug and the efficacy of the drug can be used as a traditional alternative medicine and as a Stimulant, referential information evaluated by conducting the clinical studies on patient suffering from Sex debility disease. It can be incorporated of pharmacopeial monograph and referential support for further advance research of polyherbal traditional and alternative ASU formulations of medicines.

ACKNOWLEDGMENT

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 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.

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