



PHARMACOGNOSTICAL AND HPTLC EVALUATION OF JAWARISH-E-OD TURSH: AN ANTI-EMETIC UNANI FORMULATION

Asma Sattar Khan*, Shoeb Ahmed Ansari, Sonali Sajwan, Pawan Kumar Sagar, Reesha Ahmed and
Syed Arshad Ali Hashmi

Drug Standardization Research Institute, Kamla Nehru Nagar Ghaziabad-201002.

***Corresponding Author: Asma Sattar Khan**

Drug Standardization Research Institute, Kamla Nehru Nagar Ghaziabad-201002.

Article Received on 20/11/2021

Article Revised on 09/12/2021

Article Accepted on 30/12/2021

ABSTRACT

The use of traditional medicines has increased immensely all over the world during last few decades. This growth creates major concern for health experts about the safety and quality of these medicines. Today various systems of traditional medicine exist worldwide and medicines of almost every system are being standardized in order to maintain their quality and efficacy. In Unani system of medicine, there are hundreds of single drugs and compound formulations which are used by a large population of several countries to a great advantage. One such drug Jawarish-e-Ood Tursh is very popular Unani formulation used as Digestive, Anti-emetic and Anti-bilious medicine. The drug was taken for evaluation through various scientific parameters such as microscopy, physico-chemical analysis and HPTLC fingerprinting. The drug was also analyzed for detection of heavy metals, pesticide residues, microbial load and aflatoxins to ensure its safety. The data produced in the present study will help in developing pharmacopoeial standards of Jawarish-e-Ood Tursh.

KEYWORDS: Pharmacopoeial Standardization, Microscopy, HPTLC, Pesticide residue, Heavy metals.

INTRODUCTION

There is common belief in a large section of the society that herbal medicines are safe to use. It is true to some extent until they are pure or unadulterated and free from any toxins. The quality check of these medicines is imperative as over 60% of the world population relies on traditional medicines for their primary health care which is predominantly based on plant material.^[1] Unani medicines have a long history to treat diseases; improve general health & wellbeing. The Unani formulation Jawarish-e-Ood-Tursh is very useful as digestive, anti-emetic and anti-bilious drug. It is beneficial in gastric debility, vomiting and nausea.^[3] Jawarish-e-Ood-Tursh is a classical Unani formulation categorized under the Majooniath, listed in National Formulary of Unani medicine, Part-1.^[2] Jawarish is a semi solid preparation where one or more single drugs of plant, animal or mineral origin mixed together in the powder form in a base (Qiwam) made of honey, sugar or Jaggery.

Jawarish-e-Ood Tursh is composed of fifteen different ingredients which individually have magnificent medicinal properties.^[4]

Ingredients like Ood Hindi is used for relieving loss of Appetite (Zof-e-Ishteha); Sumbul-ut-teeb is well known for Gastric Debility (Zof-e-meda); Heel Khurd is used in Nausea (Ghasayam) and Flatulent (Nafakh-e-Shikam);

Zafran is used for Hepatitis (Waram-e-Jigar); Qaranfal is used in relieving Flatulent (Nafakh-e-Shikam) and Indigestion (Bad-Hazmi); Darchini is used in Cough (Khansi), Asthma (Dam) and Diarrhoea (Ishaal); Badranj boya is effective in Paralysis (Falij) and Joint pain (Anja-e-Mufasil); Mastagi is used in loss of memory (Nisyan) and Gastric debility (Zof-e-Meda); Tabasheer is used in Vomiting due to bile (Qai-e-Safrawi) and Palpitation due to hot temperament (Khafqaan Haar); Aab-e- Leemu is well known for its digestive & appetizing properties and used in Gastric debility, Nausea, Vomiting; Arq-e-Gulab is a famous tonic for heart & brain (Muqawwi-e-qalb wa Dimag); Aab-e-Seb (Tursh) is prescribed as Cardiac tonic (Muqawwi-e-qalb) and Asl is a general tonic (Muqawwi-e-Aam).^[5]

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

MATERIAL AND METHODS

Preparation of Formulation

All the ingredients were procured from local raw drug dealer and were identified botanically using Pharmacognostical methods.^[6,7] The ingredients were further validated by comparing with the monographs available in different volumes of UPI.^[4,8]

All the ingredients of Pharmacopoeial quality were taken. The ingredients were cleaned and dried under shade to remove the moisture if any. The ingredient no. 1-8 & 10 (Table-I) were crushed separately in an Iron mortal to obtain their coarse powders. The coarse powders were further ground in a grinder to get their fine forms. The ingredient no. 9 was powdered alone with the

help of porcelain pestle & mortar by slow and light motion. The fine powders were mixed together thoroughly and sieved through mesh no. 80. The ingredients no. 11-15 were heated on low flame until the boiling started. 1% citric acid was added and mixed thoroughly to prepare the qiwan of 75% consistency. The vessel was removed from the flame and 1% Sodium benzoate was added immediately. The mixture of all the powdered ingredients (no. 1-10) were added to the qiwan and mixed thoroughly to prepare a homogenous mixture. The content was allowed to cool at room temperature. 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 I: Formulation Composition.

S. No.	Unani Name	Botanical/English Name	Part used	Qty.
1.	Ood Hindi	<i>Aquilaria agallocha</i> Roxb.	Wood	36 g
2.	Sumbul-ut-Teeb	<i>Nardostachys jatamansi</i> (D. Don.) DC.	Root	3.5 g
3.	Heel Khurd	<i>Elettaria cardamomum</i> (L.) Maton.	Fruit	3.5 g
4.	Zafran	<i>Crocus sativus</i> L.	Stigma	3.5 g
5.	Post-e-Turanj	<i>Citrus medica</i> L.	Peel	3.5 g
6.	Qaranfal	<i>Syzygium aromaticum</i> (L.) Mer. & L.M. Perry.	Bud	3.5 g
7.	Darchini	<i>Cinnamomum zeylanicum</i> Blume.	Bark	3.5 g
8.	Badranjboya	<i>Melissa parviflora</i> Benth.	Whole	3.5 g
9.	Mastagi	<i>Pistacia lentiscus</i> L.	Gum	3.5 g
10.	Tabasheer	<i>Bambusa bambos</i> (L.) Voss.	Silica crystal	3.5 g
11.	Aab-e-Seb Tursh	<i>Malus sylvestris</i> (L.) Mill.	Sour juice	230ml
12.	Arq-e-Gulab	<i>Rosa damascena</i> Herrm.	Flower	260ml
13.	Aab-e-Leemu Kaghzi	<i>Citrus aurantifolia</i> (Christm.) Swingle	Fruit	400ml
14.	Asl	<i>Apis mellifera</i> L.	Honey	430 g
15.	Qand Safaid	Sugar	As such	430 g

Microscopy

Powder Microscopy: 10-15 g of compound drug was taken and stirred carefully with hot water in a beaker; the residue was discarded and the process was repeated; the small amount of sediment was taken in a slide and mounted with glycerin; a small amount of residue was taken and treated separately with chloral hydrate, washed with distilled water and mounted with glycerine.^[6,7,9]

Physico – Chemical analysis

The physico-chemical parameters of Jawarish-e-Ood-Tursh viz. moisture content, extractive values (solubility in water and ethanol), Ash values (total ash and acid insoluble ash), pH values (1% & 10% aqueous solution), bulk density and sugar content (reducing & non-reducing) were analyzed as per standard methods.^[10,11]

High performance thin layer chromatography

After leaching out sugar from the drug, two samples of 5 g were extracted separately with 25 ml each of ethanol and chloroform by sonicating for 30 minutes. The extracts were filtered and concentrated up to 10 ml in volumetric flasks and used as such for HPTLC fingerprinting. 6µl of ethanol extract was applied on aluminum TLC plate pre-coated with silica gel 60 F²⁵⁴

(E. Marck) by employing CAMAG Linomat IV automatic sample applicator. The plate was developed up to a distance of 9 cm in twin trough glass chamber (10x10) using 10 ml of the solvent system Toluene: ethyl acetate (7:3) as mobile phase. The plate was air-dried at room temperature and observed under UV at wavelengths 254 nm and 366 nm. 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. Similarly, 8µl of chloroform extract was applied on a separate aluminum TLC plate pre-coated with silica gel 60 F²⁵⁴ (E. Marck) by employing CAMAG Linomat IV automatic sample applicator. The plate was developed up to a distance of 9 cm in twin trough glass chamber (10x10) using 10 ml of the solvent system toluene: ethyl acetate: formic acid (9: 1: 0.5) as mobile phase. Rest of the process was repeated as carried out for ethanol extract.^[12,13,14]

Quality control parameters

The herbal medicines specially Unani medicines are being used by millions of people as effective remedies assuming that they have lesser side effects or no side effects at all. People's faith in Unani medicines leads to concern over their quality assurance. Regardless of the

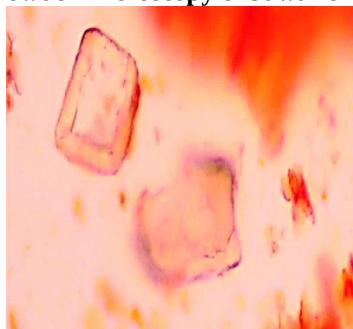
form of Unani preparation, their quality check is of greatest importance. So, the different quality control parameters like microbial loads, heavy metals, aflatoxins and pesticide residues were carried out to check the level of quality of Jawarish-e-Ood-Tursh. Estimation of microbial load was conducted as per standard method.^[15] Heavy metal analysis and aflatoxins were carried out by respective use of Atomic Absorption Spectrophotometer (LABINDIA)^[16] and HPLC (Thermo Fisher).^[18] Pesticide residues were analyzed using GC-MS system (Agilent) equipped with mass selective detector as per standard methods.^[16,17]

RESULTS AND DISCUSSION

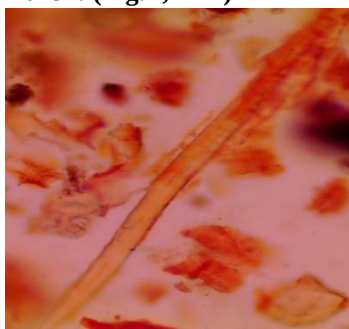
Macroscopic Description

Jawarish-e-Ood Tursh is a dark brown semi solid preparation having characteristic smell and sweet taste.

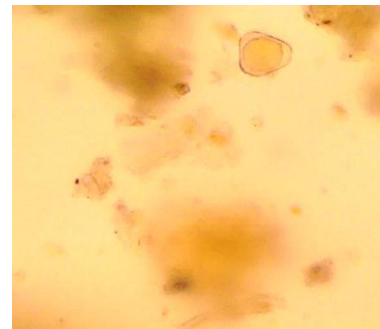
Powder microscopy of Jawarish-e-Ood Tursh: (Fig. I, A-R)



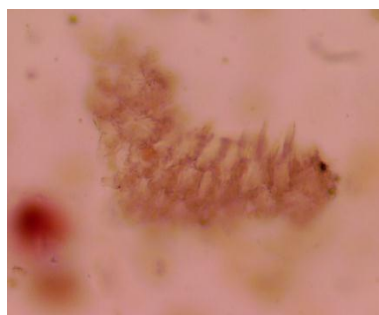
A. Darchini stone cells 20x



B. Darchini sclerenchyma fibre 20x



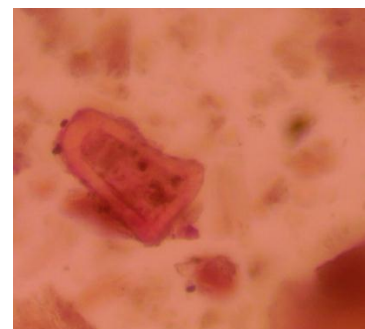
C. Qaranfal pollen grain 40x



D. Qaranfal beaded cells 20x



E. Qaranfal oil gland 20x



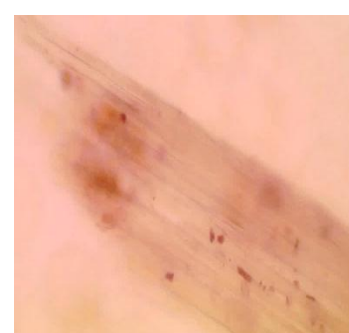
F. Qaranfal stone cells 20x



G. Sumbul-ut-teeb fibers, cork cells 10x



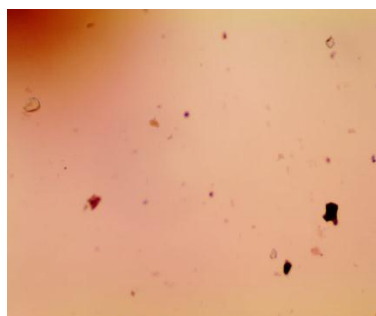
H. Sumbul-ut-teeb pitted vessels 40x



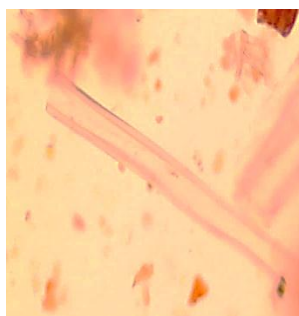
I. Turanj fibre 40x

Microscopic observation

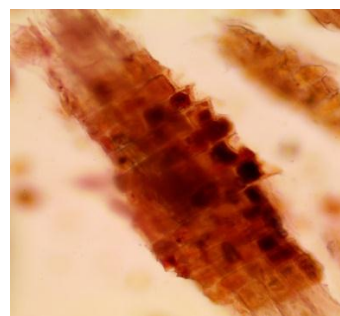
Following characters of Jawarish-e-Ood Tursh were observed under microscope: Stone cells with reddish colour, spindle shaped sclerenchyma fiber with very thick lumen (**Darchini**); Triangular shaped pollen grain, beaded shaped aerenchyma cells, oil gland, stone cells (**Qaranfal**); Fiber, cork cells, pitted vessels (**Sumbul-ut-teeb**); Fibre, calcium oxalate crystals (**Turanj**); Wood fibre having narrow lumen, medullary rays (**Ood hindi**); Annular vessels, many septate fibres (**Badranjboya**); Fragment of perisperm, cells of seed coat (**Heel khurd**); Smooth spherical pollen grains, annular and spiral vessels (**Zafran**). (Fig. I, A-R)



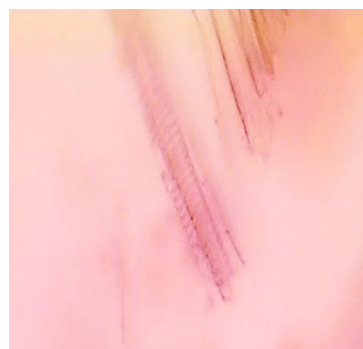
J. Turanj crystals 40x



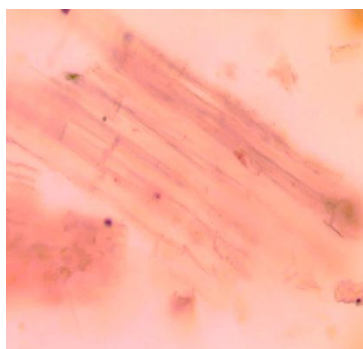
K. Ood hindi wood fibre 20x



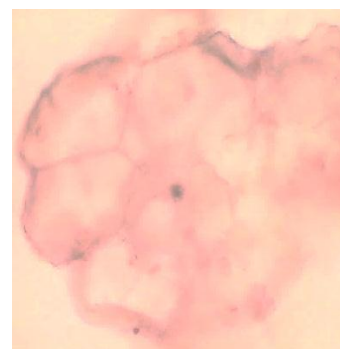
L. Ood hindi medullary rays 20x



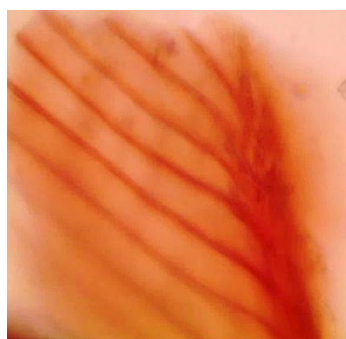
M. Badranjboya annular vessels 20x



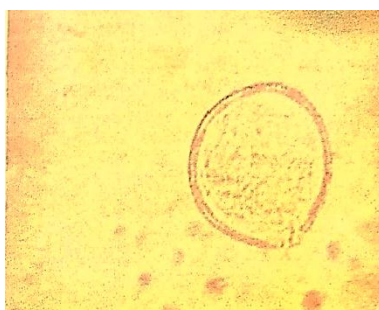
N. Badranjboya septate fibre 20x



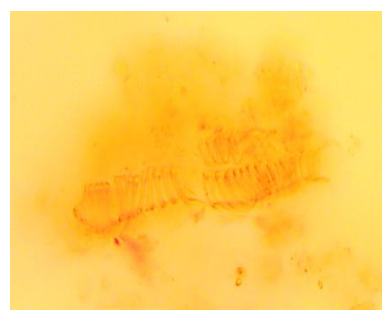
O. Elaichi fragment of perisperm 40x



P. Elaichi cells of seed coat 40x



Q. Zafran Pollen grains 40x



R. Zafran spiral vessels 40x

Physico-Chemical analysis

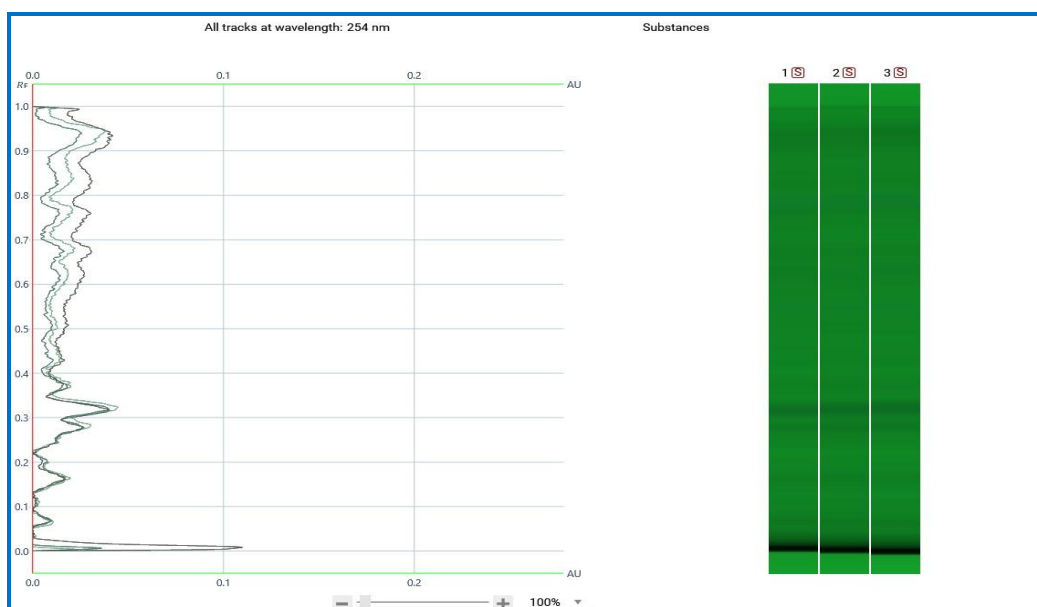
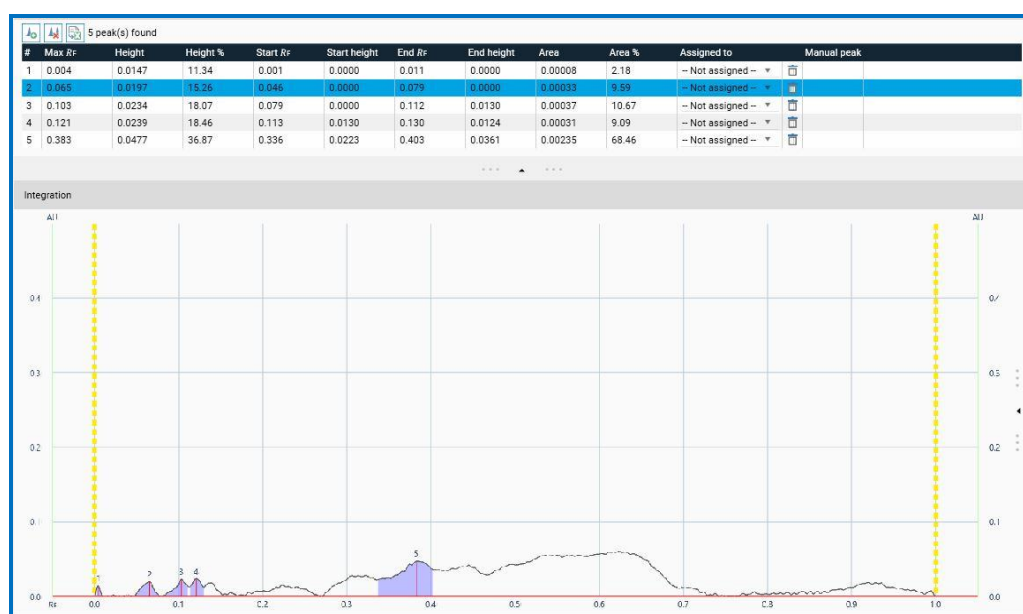
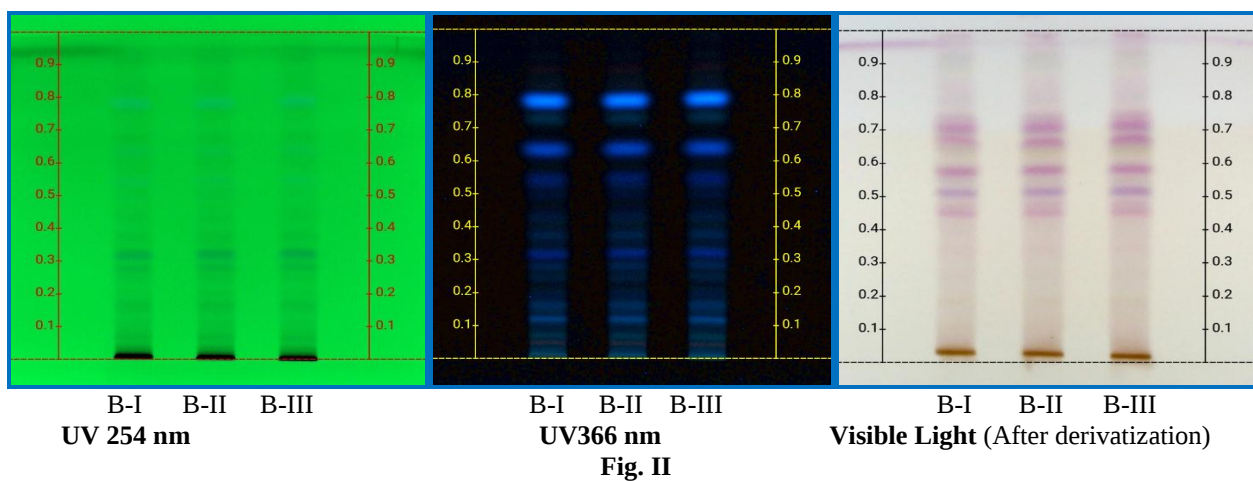
The physico-chemical data of the drug Jawarish-e-Ood-Tursh are shown in Table-II. The quantitatively evaluated data revealed that the moisture content of the drug ranged between 22.77 & 23.62 which is appropriate in case of Jawarish. The total ash content is not more than 0.65% and acid insoluble ash remains very low i.e. 0.30% which shows the negligible amount of siliceous matter in the drug sample. The water extractive values turned out to be on higher side, ranged between 70.35 – 71.70% which marks the presence of inorganic constituents in the drug sample and the ethanol soluble extractive values were also high and ranged between 52.22 - 53.58 which indicates the extraction of polar constituents. The aqueous extract of the drug was acidic in nature as pH value falls in the range of 2.5-3.5.

HPTLC Profile

HPTLC fingerprinting is efficient, faster, reliable and reproducible technique for the analysis of complex mixtures of natural products. It is a convenient tool for

identification of crude drugs. HPTLC images of ethanol & chloroform extracts of Jawarish-e-Ood Tursh were observed under UV254 nm, UV 366 nm and under visible light after derivatization. All the batches of Jawarish-e-Ood Tursh show similar colourful bands and similar R_f values. Moreover, their densitograms are almost super imposed on each other. This shows batch to batch consistency of the drug. (Fig. II, III).

HPTLC of *Ethanol* extracts of Jawarish-e-Ood Tursh



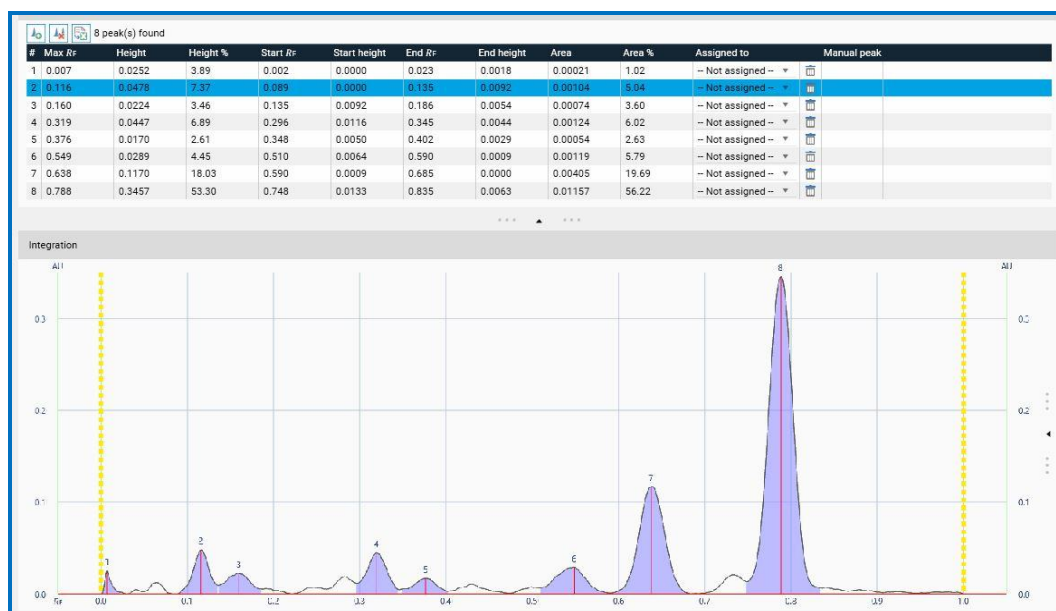


Fig. II(c) HPTLC fingerprint profile of ethanol extract of Jawarish-e-Ood Tursh at 366 nm.

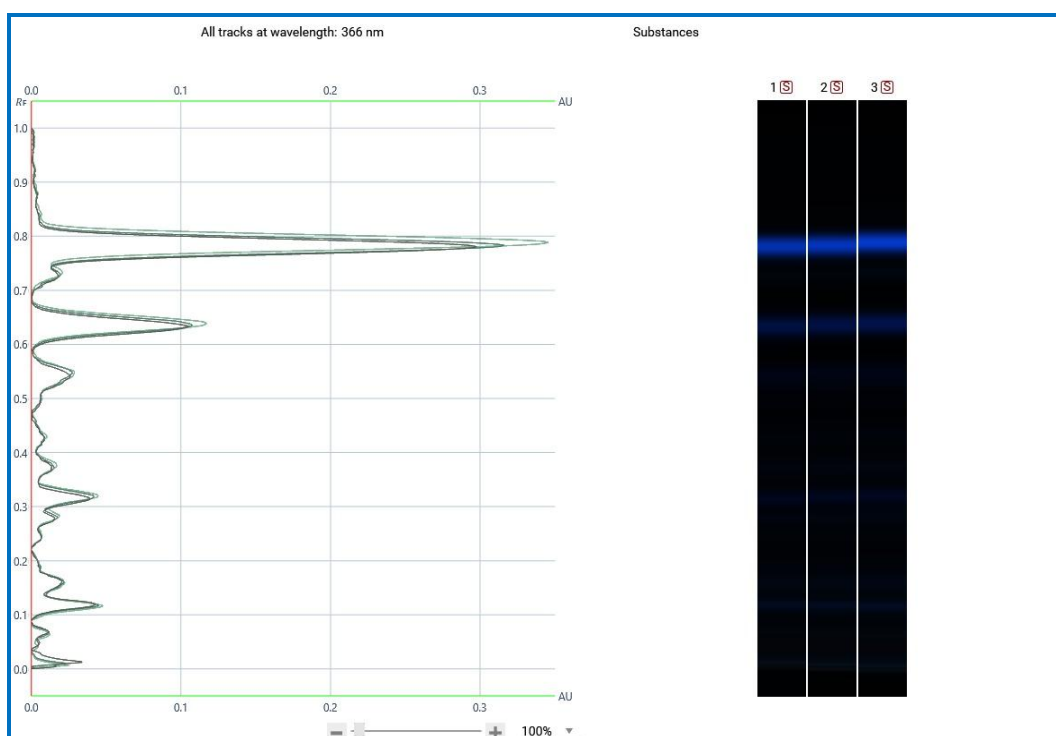


Fig. II(d) HPTLC densitometry chromatogram of ethanol extracts of Jawarish-e-Ood Tursh (03 batches) at 366 nm.

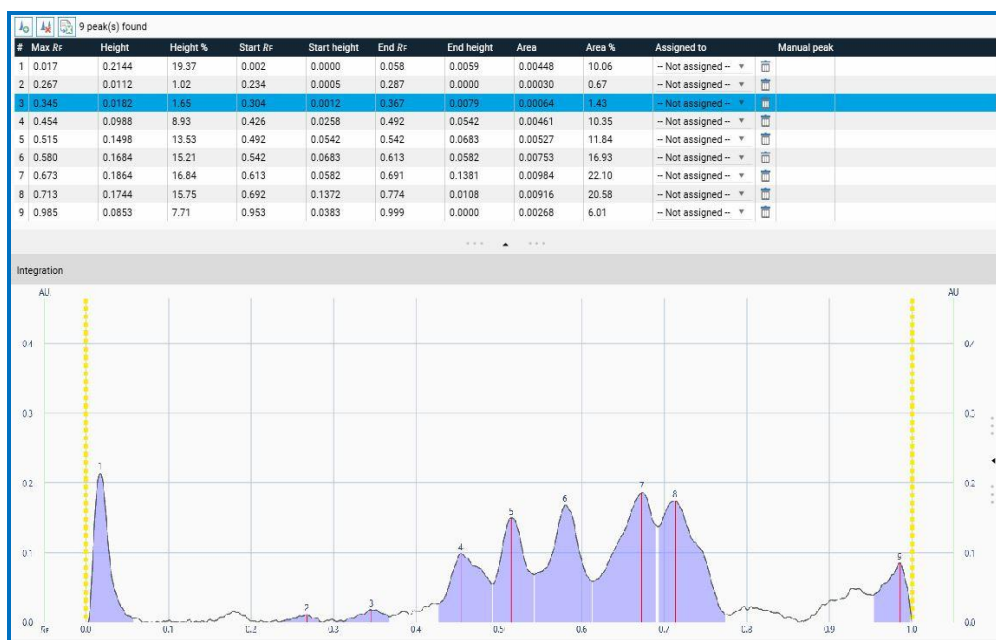


Fig. II(e) HPTLC fingerprint profile of ethanol extract of Jawarishe-e-Ood Tursh under white light after derivatization.

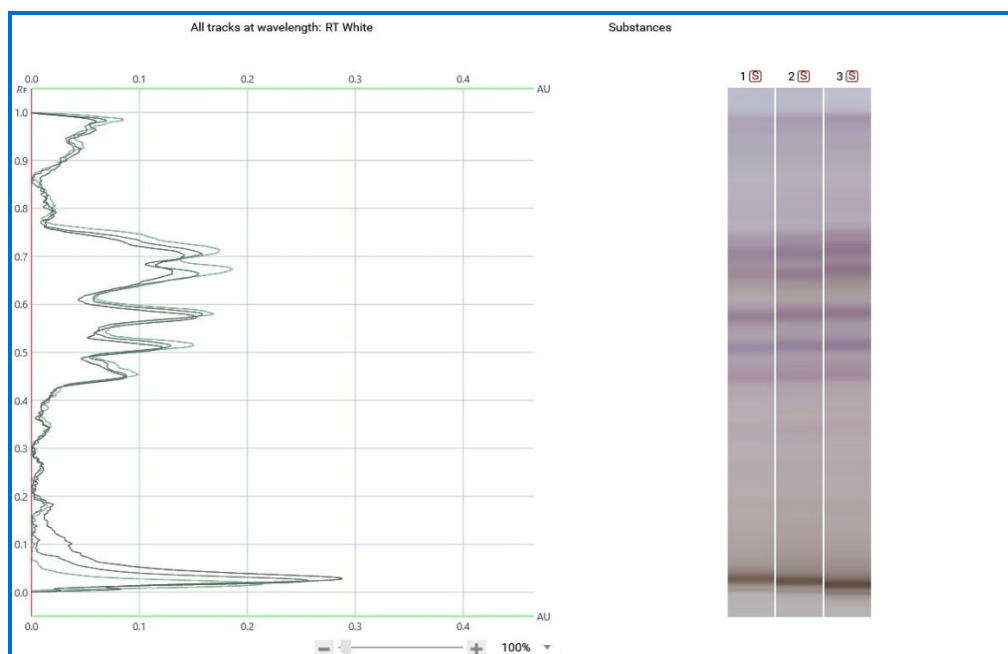


Fig. II(f) HPTLC densitometry chromatogram of ethanol extracts of Jawarishe-e-Ood Tursh (03 batches) under white light after derivatization.

HPTLC of *Chloroform* extracts of Jawarish-e-Ood Tursh

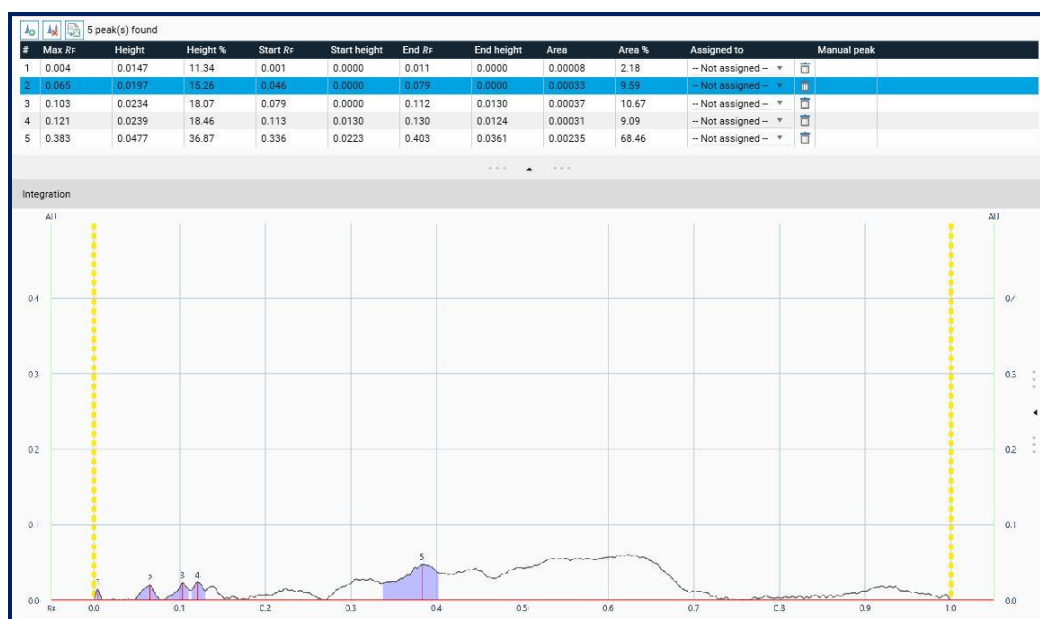
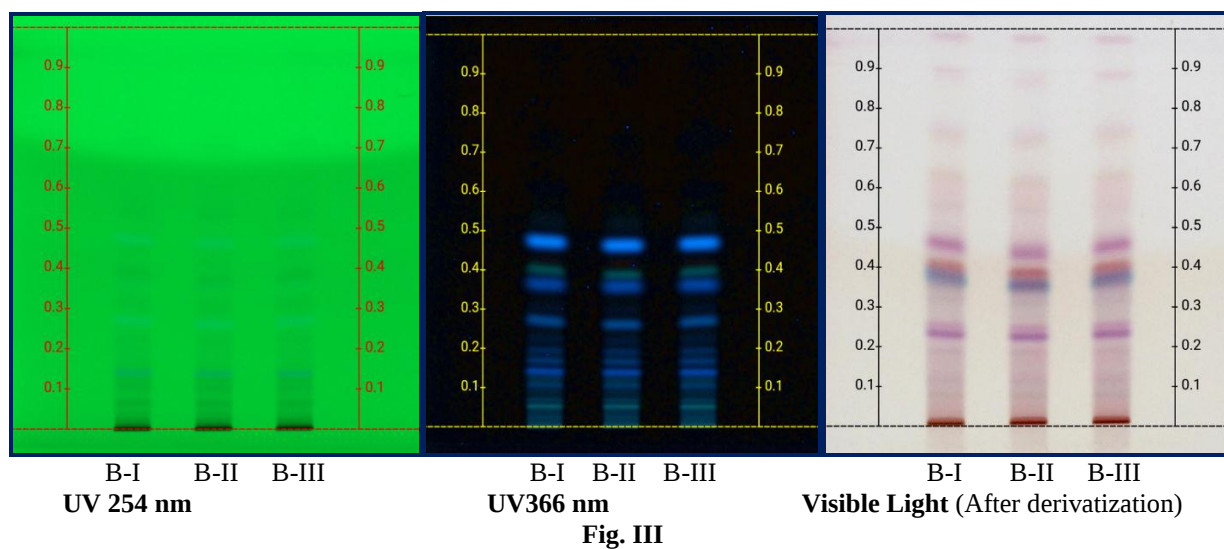


Fig. III(a) HPTLC fingerprint profile of chloroform extract of Jawarish-e-Ood Tursh at 254 nm.

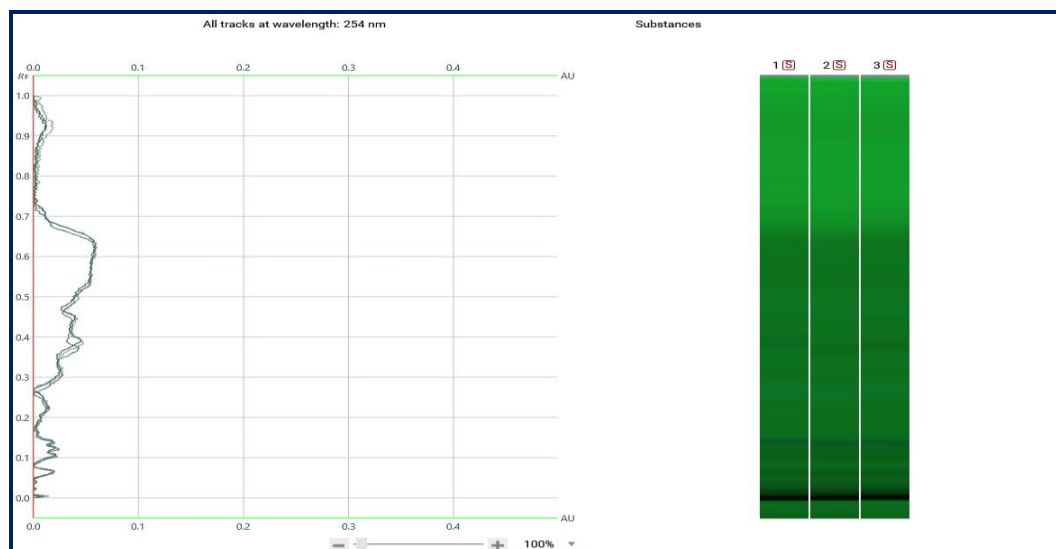


Fig. III(b) HPTLC densitometry chromatogram of chloroform extracts of Jawarish-e-Ood Tursh (03 batches) at 254 nm.



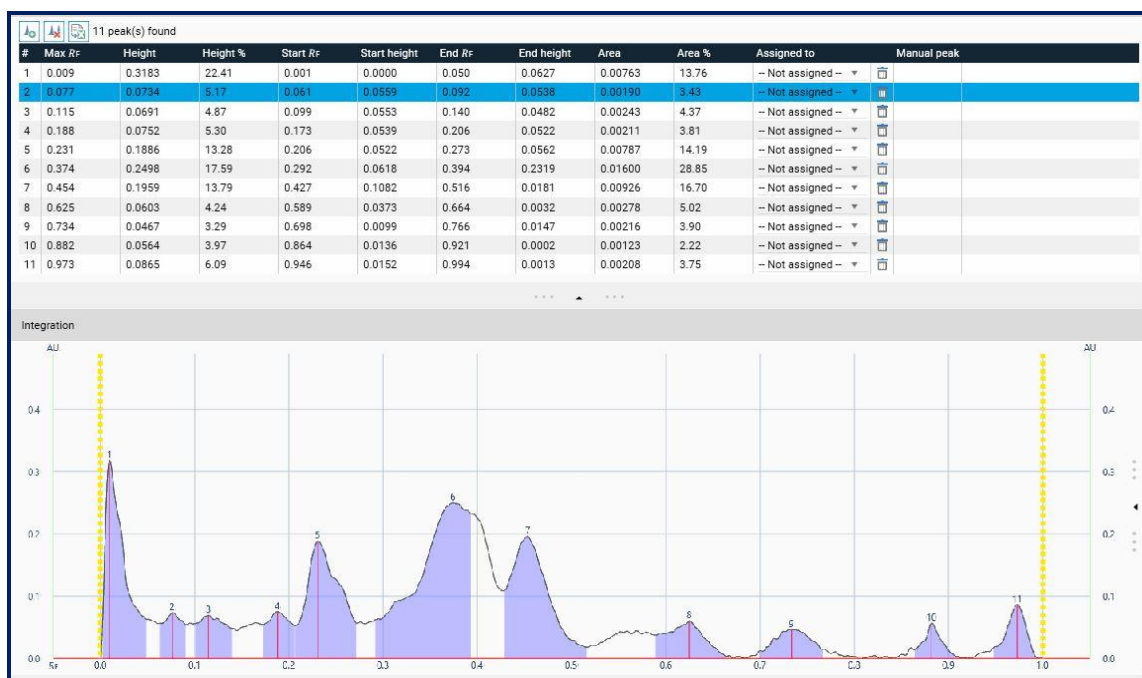


Fig. III(e) HPTLC fingerprint profile of chloroform extract of Jawarish-e-Ood Tursh under white light after derivatization.

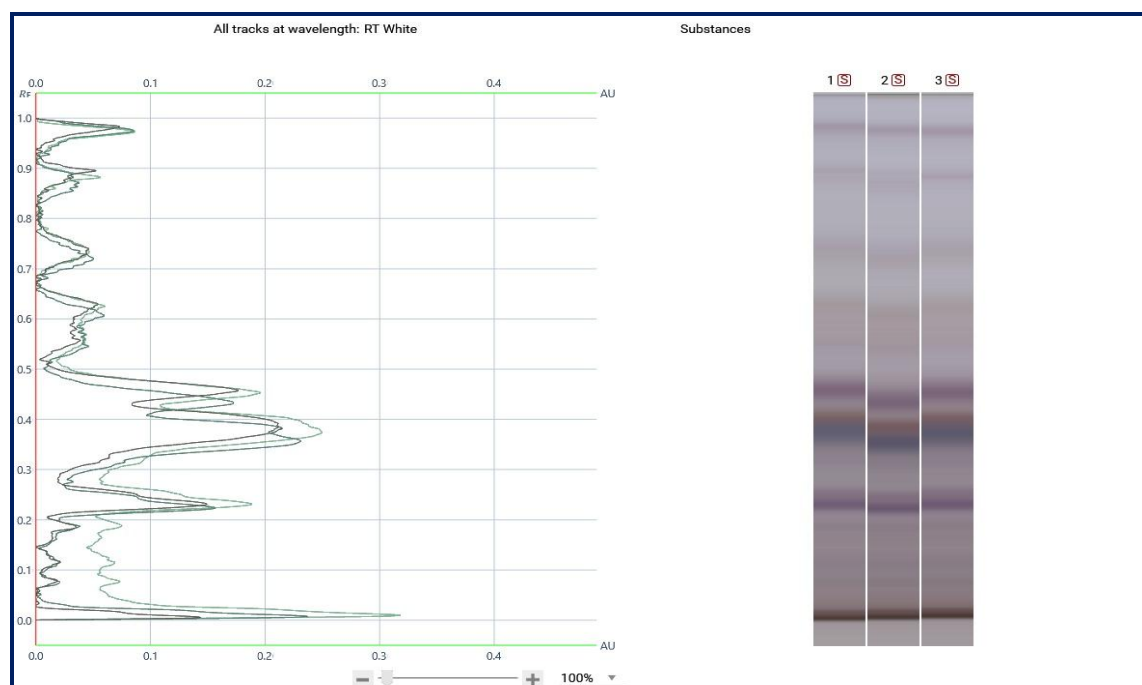


Fig. III(f) HPTLC densitometry chromatogram of chloroform extracts of Jawarish-e-Ood Tursh (03 batches) under white light after derivatization.

Quality Control Parameters

Heavy metal analysis

The results of heavy metal estimation are given in Table III. Heavy metals are toxic or poisonous to human health even at low concentrations and may cause many deadly diseases. The heavy metal content in Jawarish-e-Ood Tursh was found to be below detection limit which indicated that the drug was free from heavy metal contamination.

Microbial load

Traditional medicines are largely spoiled due to microbial growth. Prevention of microbes can extend the shelf life of the drug. The estimation of microbial load 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 are shown in Table IV which indicate that the drug is safe for human consumption.

Aflatoxins

The results of aflatoxins analysis of the drug are given in Table V. 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 aflatoxin contents (B1, B2, G1, and G2) in Jawarish-e-Ood Tursh.

Pesticide residues

The results of pesticide residues are given in table VI. Excessive use of pesticides to protect the crops from insects or diseases has created hazardous conditions for human food chain. They have to be checked not only in food items but also in traditional medicines. In order to estimate the pesticide residues, the drug was analyzed on GC-MS. The results indicated that the drug is free from pesticide residues and safe for use.

CONCLUSION

Currently there is limited scientific evidence for the safety, efficacy and authenticity of traditional medicines which usually does not provide standards for assertive identification. Thus, standardization is vital for evaluation of the quality of traditional medicines. Jawarish-e-Ood-Tursh was evaluated through pharmacopoeial parameters which certainly provide an assurance of quality of the drug. All the physico-chemical parameters, quality control parameters viz. heavy metals, microbial load, aflatoxins and pesticidal residue were found within permissible limits which conspicuously show that the drug Jawarish-e-Ood Tursh is free from harmful toxins and can be used safely. Hence, the present study contains high significance to ensure authenticity and quality of the drug.

ACKNOWLEDGEMENT

The authors are extremely grateful to the Director-General, CCRUM, New Delhi for his fortifying encouragement and providing necessary research facility. We are also thankful to DSRU (CCRUM), Chennai for providing necessary assistance.

Table II: Physico-chemical analysis.

Parameters	Values
Ethanol soluble matter (%)	52.22 – 53.58
Water soluble matter (%)	70.35 – 71.70
Loss in weight on drying at 105 ⁰ C (%)	22.77 - 23.62
Total ash (%)	0.52 – 0.65
Acid insoluble ash (%)	0.24 – 0.30
pH of 1% aqueous solution	3.58 – 3.62
pH of 10% aqueous solution	2.80 – 2.86
Bulk density	1.4554 - 1.4676
Reducing sugar	59.40 – 61.45
Non-reducing sugar	11.36 – 12.80

Table III: Heavy Metals Estimation.

S. No.	Element	Values	WHO Limits for internal use
1.	Lead	< LOD	10 ppm
2.	Cadmium		0.3 ppm
3.	Arsenic		3.0 ppm
4.	Mercury		1.0 ppm

Table IV: Microbial load.

S. No.	Microbial contaminant	Values	WHO Limits for internal use
1.	Total Bacterial load	1 x10 ¹ cfu/gram	1x10 ⁵ cfu/g
2.	Total Fungal count	< 1 cfu/ gram	1x10 ³ cfu/g
3.	Enterobacteriaceae	Absent	NIL
4.	<i>Escherichia coli</i>	Absent	NIL
5.	<i>Salmonella spp.</i>	Absent	NIL
6.	<i>Staphylococcus aureus</i>	Absent	NIL
7.	<i>Pseudomonas aeruginosa</i>	Absent	NIL

Table IV: Aflatoxins.

S. No.	Aflatoxin	Result
1.	B ₁	< LOD
2.	B ₂	
3.	G ₁	
4.	G ₂	

Table VI: Pesticide residue.

S. No.	Pesticide Residues	Result
1.	Alachlor	< LOQ
2.	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	
3.	Azinophos-methyl	
4.	Bromopropylate	
5.	Chlordane (cis, trans and oxychlordane)	
6.	Chlorfenvinphos	
7.	Chlorpyrifos	
8.	Chlorpyrifos-methyl	
9.	Cypermethrin (and isomers)	
10.	DDT (all isomers, sum of p,p'-TDE (DDD) expressed as DDT)	
11.	Deltamethrin	
12.	Diazinon	
13.	Dichlorvos	
14.	Dithiocarbamates (as CS ₂)	
15.	Endosulphan (sum of isomers & Endosulphan sulphate)	
16.	Endrin	
17.	Ethion	
18.	Fenitrothion	
19.	Fenvalerate	
20.	Fonofos	
21.	Heptachlor (sum of Heptachlor & Heptachlor epoxide)	
22.	Hexachlorobenzene	
23.	Hexachlorocyclohexane isomer (other than γ)	
24.	Lindane (γ – Hexachlorocyclohexane)	
25.	Malathion	
26.	Methidathion	
27.	Parathion	
28.	Parathion methyl	
29.	Permethrin	
30.	Phosalone	
31.	Piperonyl butoxide	
32.	Pirimiphos methyl	
33.	Pyrethrins (sum of isomers)	
34.	Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	

REFERENCES

- World Health Organization. WHO global report on traditional and complementary medicine. World Health Organization, Geneva, Switzerland, 2019.
- Anonymous. National formulary of Unani Medicine, Part-I, CCRUM, Ministry of health and family welfare, Govt. of India, New Delhi, 2006; 103.
- Kabiruddin H.M. Bayaz-e-Kabir, Vol. II, CCRUM, Ministry of Health & family welfare, Govt. of India, New Delhi, 2006; 30.
- Anonymous. Unani Pharmacopoeia of India, Part I, Vol. I, III, V & VI, Ministry of health and family welfare, Govt. of India, p.34, 84, 26, 70 (Vol. I); 31, 109 (Vol. III); 50 (Vol. V) & 101 (Vol. VI).
- Kabiruddin M. (YNM), Makhzan-ul-Mufridaat Al-Marooif Khawaas-ul-Advia, Ainaz publication House, Kuncha Chalan, Daryaganj, New Delhi, 2019; p.84, 356, 85, 317, 517, 279, 111, 546, 392, 389, 521, 490 and 371.
- Wallis TE. Textbook of Pharmacognosy, 5th ed. CBS Publishers & Distributors Pvt. Ltd., New Delhi, 2005; 578: 493-494.
- Trease G.E. and Evans W.C. Pharmacognosy. Baillière Tindall, London, 1989; 13: 5-9.

8. Anonymous. Unani Pharmacopoeia of India Part II, Vol. I, Ministry of Health and family welfare, Govt. of India, 2009; 82: 256.
9. Johansen, D.A. Plant micro techniques, Mc-Graw Hill book company Inc. New York and London, 1940; 13: 65-105.
10. Anonymous. Physicochemical standards of Unani formulations, Part-II, CCRUM, Govt. of India, New Delhi, 1987; 2: 268-281.
11. Anonymous. Quality control methods for medicinal plant materials, World Health Organization, Geneva, 1998; 28-33.
12. Wagner H. and Bladt S. Plant Drug Analysis – A Thin Layer Chromatography Atlas, Springer Verlag, Germany, 1996; 1-3: 293-303.
13. Sethi P.D. High Performance Thin Layer Chromatography, 1st ed., Vol. X, CBS Publishers and Distributors, New Delhi, 1996; 4-20.
14. Stahl E. Thin Layer Chromatography – A laboratory handbook. George Allen and Unwin Ltd., London, 1996; 900.
15. Anonymous. WHO Guidelines for assessing the quality of herbal medicines with reference to contaminants and residues, World Health Organization, Geneva, 2007; 27-28: 55-68.
16. Anonymous. Official Method of Analysis, Horwitz, W, Latimer GW (ed) 18th ed. AOAC International Maryland, 2005; 3:10-17, 10: 8-23.
17. Anonymous. Official methods of analysis, Association of official Analytical chemist (AOAC) Arlington, USA, 2000; 17: 38-60.
18. Anonymous. Official analytical methods of the American spices Trade Association (ASTA) Inc. 4th ed. New Jersey, USA, 1997.