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

ISSN 2349-8870
Volume: 2
Issue: 5
402-411
Year: 2015

PHARMACOPEIAL STANDARD DEVELOPMENT, HPTLC. FINGERPRINTING AND PHYSICOCHEMICAL RESEARCH STUDIES OF UNANI ANTI-PARALYTIC DRUG MAJOON-E-SEER ALWI KHANI

[1*]Pawan Kumar Sagar, [2]Munawwar Husain Kazmi, [3]Javed Inam Siddiqui, [4]M. A. Rasheed N

[1*]Research Officer (Chemistry) and DSRU. In-charge, DSRU. Central Research Institute of Unani Medicine, (CCRUM., AYUSH.) Hyderabad.-500038, T.S.

[2]Dy. Director In-charge, Central Research Institute of Unani Medicine, (CCRUM., AYUSH.) Hyderabad.-500038, T.S.

[3]Research Officer (Unani), Central Research Institute of Unani Medicine, (CCRUM., AYUSH.) Hyderabad.-500038, T.S.

[4]Research Assistant (Chemistry), DSRU. Central Research Institute of Unani Medicine, (CCRUM., AYUSH.) Hyderabad.-500038, T.S.

Article Received on 08/08/2015 Article Revised on 29/08/2015 Article Accepted on 20/09/2015

***Correspondence for Author**
Pawan Kumar Sagar
Research Officer (Chemistry) and DSRU. In-Charge, DSRU. Central Research Institute of Unani Medicine, (CCRUM., AYUSH.) Hyderabad.-500038, T.S.

ABSTRACT

Pharmacopeial Standard development, HPTLC. Fingerprinting and Physicochemical research studies of Unani Anti-paralytic drugs Majoon-e-Seer Alwi Khani a classical formulation used in the treatment of Falij (Paralysis) and Laqwa(Facial Paralysis) and other Neurological disorders. Three drug samples of this compound formulation were taken into the studies which prepared in the Pharmacy at CRIUM., Hyderabad(Under CCRUM. Ministry of AYUSH.) by employing authenticated standard methods. The quality control & quality assurance studies were conducted in accordance to the WHO., AOAC., IPC. and UPC., approved guidelines. During these studies the physicochemical data showed that the drug samples contained foreign matter %, w/w., moisture /LOD. %, w/w. (not more than-7.41). and ash contained %.,w/w.(not more than-1.59), acid in-soluble ash %.,w/w.(not more than-0.10), soluble extract in water %.,w/v. (not

less than-65.06) and in alcohol %.,w/v., (not less than-5.62) pH (1% Aq. Solution-6.31), pH (10 % Aq. Solution-6.01),Reducing sugar (33.98), Non-reducing sugar (5.63) were assessed in Majoon-e-SeerAlwiKhani. HPTLC studies of aqueous and alcohol extracts of both drug samples showed various spots at 366nm and 254nm (UV. region),under exposed to Iodine vapours, derivatized with 5% Methanolic sulphuric acid obtained with equate, best separation using selected suitable solvent system of mobile phase. The quality control studies results revealed the absence of hazardous and toxic contamination from the drug sample. Moreover the obtained research studies data and comparative screening will provide the referential supportive information in the development of pharmacopeial standard monographs and identification, reinvestigation, quality assurance and pharmacovigilance of the drug.

KEYWORDS: Majoon-e-Seer Alwi Khani, Anti-paralytic, development of pharmacopeial standards, physicochemical, comparative screening, quality control and quality assurance.

INTRODUCTION

The research study drug Majoon-e-Seer Alwi Khani is frequently recommended as Anti-paralytic drugs Unani system of medicine. In most of the Asian, European and Arabian countries it is used since ancient time as traditional and alternative medicines. The drug has used and reported to Action wise Muqawwi-e-Asab (Nervine tonic) and Muhrrik-e-Asab(Nervine Stimulant) action and therapeutic use in Tasammum (Intoxication), Falij (Paralysis or Hemiplegia), Laqwa (Facial Paralysis) and other Neurological disorder etc. Majoon-e-Seer Alwi Khani is dark brown coloured semi-solid preparation with agreeable, aromatic odour and sweetish and slightly tending bitter taste. Majoon-e-Seer Alwi Khani was reported bioactive to contained phytochemical constituents as Alkaloids -0.39%, Tannins - 0.07%, Crude fibers - 2.74%, and total nitrogen 0.642% also contained volatile oil - 0.018%.[1-2,7]

MATERIAL AND METHODS

Procurement of the plant material

Raw drug ingredients were procured from national raw drug vendors and from Pharmacy CRIUM. Hyderabad. The Collected drug samples were botanically and pharmacognosticaly identified by Survey of Medicinal Plant Unit researchers, botanists and Drug Standardization Research Units researchers of CRIUM., Hyderabad. The dried crude drug samples were procured and after proper Quality control and Quality assurance take required quantity of

pure quality of each composition drug as per batch size of standard formula for preparation of formulated compound drug.

Description

Dark brown colored semi-solid preparation with agreeable, aromatic odour and sweet slightly tending bitter taste.

Identification

Macroscopic

Majoon-e-Seer Alvi Khani is dark brown colored semi solid preparation with sweetish bitter taste and aromatic odour. The samples were spreaded in a petri dish and observed. No filth, fungus or objectionable extraneous matters were found in the samples.

Definition

Majoon-e-Seer Alwi Khani is a semi solid preparation made of ingredients in the formulation composition quantity given below like.[3,7-8]

Method of preparation

Take all the formulation composition Majoon-e-Seer Alwi Khani ingredients of pharma copoeial quality.Ingredients 2 to 7 are boiled in 3 Liters of water to obtain 1 liter of decoction. Seer Taza is then boiled with this decoction till it gets soft. Then Sheer-e-Gao is added and boiled till the decoction evaporate. To it Raughan Zard is added and boiled for same time. Asal is then added and Take all the ingredients of pharmacopoeial quality. During preparation Clean, dry and powder the ingredients number 1 and 11 to 23 and pass through sieve number 80.Grind the ingredient number 24 and 25 separately in mortar and pestle for 4 hours till it becomes fine powder. followed by adding the ingredient number 24 and 25, mix thoroughly on slow heat and prepare the final qiwam, At boiling stage add 0.1% citric acid, mix well and heat gently, keep the heating continue till it attains the three tar consistency to prepare the 74-76%, (Brix) consistency of qiwam. Then remove the container from fire and while hot add the fine powders of ingredient number 1 and 11 to 23 along with 0.1 % of sodium benzoate and mix thoroughly to get the homogenous product. Allow to cool to room temperature. Pack it in tightly closed food grade containers to protect from light and moisture. brought to the required consistency to prepared the majoon as used.

Sample 1, -A1 **Sample 2,-A2** **Sample 3,-A3**

Figure-1, Over view of Majoon-e-Seer Alwi Khani formulated compound drug samples.

Formulation Composition of Majoon-e-Seer Alwi Khani formulated compound drug.

Sr. N0.	Ingredients, Local Unani Name	Botanical name	Part Used	Quantity Used
01.	Seer Taza(Lahsun)	*Allium sativum* Linn.	Bulbs	500.0 gm.
02.	Gul-e-Gaozaban	*Borogo officinalis* Linn.	Flowers	100.0 gm.
03.	Badranjboya	*Nepetahindostana*(Roth.) Haines	Leaves	100.0 gm.
04.	Bisfayej	*Polypodiumvulgare* Linn.	Roots, rhizomes	50.0 gm.
05.	Halela Siyah	*Terminalia chebula* Retz	Unripe Fruits	50.0 gm.
06.	Post-e-Halela Kabili	*Terminalia chebula* Retz	Mature Fruits	50.0 gm.
07.	Inab-us-salab (Mako)	*Solanum nigrum* Linn.	Fruits	50.0 gm.
08.	Sheer-e-Gao	Cows milk	Milk	1000.0 gm.
09.	Raughan Zard	Pure ghee	Milk fat	500.0 gm.
10.	Asal or Qand Safaid	Honey/ Sugar	Fructose /Sucrose	1000.0 gm.
11.	Zanjabeel	*Zingiber officinals* Roxb.	Rhizomes	25.0 gm.
12.	Filfil Siyah	*Piper nigrum* Linn.	Fruits	25.0 gm.
13.	Filfil Safaid	*Piper nigrum* Linn.	Fruits	25.0 gm.
14.	Filfil Daraz	*Piper longum* Linn.	Fruits	25.0 gm.
15.	Qaranful	*Syzygium aromaticum* (L). Merr.& Perry	Flower buds	25.0 gm.
16.	Saleekha	*Cinnamomumcassia* Blume	Stem barks	25.0 gm.
17.	Kababchini	*Piper cubeba* Linn. f.	Fruits	25.0 gm.
18.	Khulanjan	*Alpinia galanga* Willd.	Rhizomes	25.0 gm.
19.	Behman Surkh	*Salvia haematodes* Linn.	Root	25.0 gm.
20.	Behman Safaid	*Centaurea behen* Linn.	Root	25.0 gm.
21.	Shaqaq-ul-Misri	*Pastinaca secacul* Linn.	Rhizomes	25.0 gm.
22.	Gul-e-Babuna	*Matricariachamomilla* Linn.	Flowers	25.0 gm.
23.	Marzanjosh	*Olanum vulgora* Linn.	Whole herb	25.0 gm.
24.	Ambar Ash-hab	Ambar grasea	Sea animal origin	5.0 gm.
25.	Zafran	*Crocus sativus* Linn.	Style and Stigma	5.0 gm.

Physicochemical screening

Physicochemical screening was carried out under the following parameters like foreign matter, %, total ash contained, % at 450°C, acid insoluble ash, % at 550°C, and loss on drying at 105°C. water and alcohol soluble extractive matter, % were carried out as per IPC. approved standard methods.[3,7-8]

HPTLC. (TLC.) Analysis

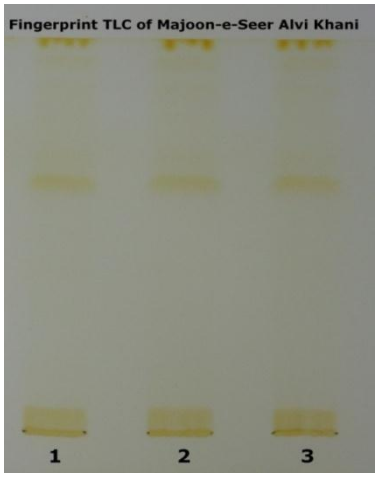
Extract 2 g of sample with 20 ml of ethanol separately by refluxing on a water bath for 30 min. The extractives were filtered (through Whatman N0.1 filter paper) and concentrated made up to 5ml in standard flask separately. Made / Make of HPTLC. Instrument used Desaga Sarstedt Gruppe (Germany).Concentrate to 5 ml and carry out the thin layer chromatography. Apply the ethanol extract on TLC plate.

TLC. Developing method

Alcohol extracts was applied on pre-coated aluminium plates silica gel $60F_{254}$ TLC plate (Merck, KgaA, Germany) as absorbent stationary phase and developed the plates using the solvent systems Toluene: Ethyl acetate: Methanol(7:2:1) as mobile phase respectively. Develop the plate as Alcoholic extract was spotted & applied 0.5 to 2.0 µl. concentrated drug extract on prepared Aluminium sheet pre coated with silica gel merck $60F_{254}$ as stationary phase. Separated and Developed plate was Dried at 90-100°C temperature in hot air oven showed three spots under UV 366nm at Rf values 0.49 (blue), 0.61 (Blue) and 0.70(blue); and under Iodine vapours showed three spots at Rf values 0.04, 0.65, 0.99 (All brown); and under visible region after derivatizing with 5% methanolicsulphuric acid showed one spot at Rf value 0.65(light purple).[4-7]

Quality Control

Quality control was taken under the WHO, AOAC guidelines and IPC.approved format.[4-5,7,9]



At 366nm Exposed under iodine vapour

After derivatized with 5% Methanolic sulphuric acid.

Figure -2. HPTLC. view of Majoon-e-Seer Alwi Khani Alcoholic extracts of A1 as 1, A2 as 2, A3 as 3 samples., Detector develop observation at 366 nm. UV. Region: Solvent System: (Toluene: Ethyl acetate: Methanol, (7:2:1) ratio).

Table-I. Uses and dose form of Majoon-e-Seer Alwi Khani formulated classical drugs.

Action with modern equivalents	Muqawwi-e-Asab (Nervine tonic), Muhrrik-e-Asab (Nervine Stimulant)	**References**
Therapeutic uses with modern equivalents	Tasammum (Intoxication), Faliz (Paralysis or Hemiplegia), Laqwa (Facial Paralysis), Rasha (Tremers)	[1,7]
Recommended dose form	5 to 10 gm.	
Mode of Administration	Oral	

Table-II. Physicochemical parameters standardization.

Parameters Analyzed	Sample 1, -A1	Sample 2, -A2	Sample 3, -A3	Mean value
Loss on drying % at 105°C temp.	6.34	7.27	8.62	7.41
Total ash%, w/w.	1.65	1.54	1.60	1.59
Acid insoluble ash%, w/w.	0.11	0.10	0.11	0.10
pH (1% Aq. Solution)	6.31	6.30	6.32	6.31
pH (10 % Aq. Solution)	6.02	6.01	6.02	6.01
Alcohol Sol. Extractive Matter, %,w/v.	5.57	5.62	5.69	5.62
Water Sol. Extractive Matter, %,w/v.	63.68	66.26	65.24	65.06
Reducing sugar	34.24	33.57	34.14	33.98
Non-reducing sugar	5.29	5.93	5.68	5.63

Table-III. Estimation of Microbial load and contamination.

Sr. N0.	Parameters Analyzed	Results			WHO. & API. Limits
		Sample1, - A1	Sample2, - A2	Sample3, - A3	
1.	**Total Bacterial Count**	$40X10^2$	$40X10^2$	$40X10^2$	10^5 cfu/gm.
2.	**Total Fungal Count**	$36X10^2$	$37X10^2$	$36X10^2$	10^3cfu/gm.
3.	***Salmonella Spp.***	Absent	Absent	Absent	Nil
4.	***Staphylococcus aureus***	Absent	Absent	Absent	Nil
5.	***Escherichia coli***	Absent	Absent	Absent	Nil

Table-IV. Estimation of Heavy metals contamination.

Sr. N0.	Parameters Analyzed	Results			WHO. & API. Limits
		Sample1, - A1	Sample2, - A2	Sample3, - A3	
1.	**Arsenic**	Not detect	Not detect	Not detect	3.0 ppm.
2.	**Cadmium**	Not detect	Not detect	Not detect	0.3 ppm.
3.	**Lead**	Not detect	Not detect	Not detect	10.0 ppm.
4.	**Mercury**	Not detect	Not detect	Not detect	1.0 ppm.

Table-V. Estimation of Aflatoxins contamination.

Sr. N0.	Parameters Analyzed	Results			WHO. & API. Limits
		Sample1, - A1	Sample2, - A2	Sample3, - A3	
1.	**B1 Aflatoxin**	Not detect	Not detect	Not detect	0.5 ppm.
2.	**B2 Aflatoxin**	Not detect	Not detect	Not detect	0.1 ppm.
3.	**G1 Aflatoxin**	Not detect	Not detect	Not detect	0.5 ppm.
4.	**G2 Aflatoxin**	Not detect	Not detect	Not detect	0.1 ppm.

Table-VI. Rf. Value of Majoon-e-Seer Alwi Khani Alcoholic extract.

Rf. Values of separated spots of A1,A2 and A3 samples		
Under UV. Region at 366nm	**Under exposed to Iodine vapours**	**Under visible region after derivatizing with 5% methanolic sulphuric acid**
0.49, (Blue)	0.09 ,(Brown)	0.65 ,(Light purple)
0.61, (Blue)	0.65 ,(Brown)	
0.70, (Blue)	0.99 ,(Brown)	
Where: A1 as 1, A2 as 2, A3 as 3 samples of Alcoholic Majoon-e-Seer Alwi Khani extracts.		

RESULT AND DISCUSSION

The study drug Majoon-e-Seer Alwi Khani is frquentely and widely used for the treatment Action wise as it has Muqawwi-e-Asab (Nervine tonic) and Muharrik-e-Asab (Nervine stimulant) and therapeutic used wise for the treatment of Tasammum (Intoxication),Falij (Paralysis) and Laqwa(Facial Paralysis) and other Neurological disorders. The drug is mostly available and sold in as Unani classical Drug and Unani proprietary medicine, Drug market of the country. Physicochemical parameters, High Performance Thin Layer Chromatography (HPTLC.) analysis, Quality control, Quality assurance parameters of the semi soiled formed Unani drug terminology Majoon-e-Seer Alwi Khani and this result are described and discussed as follows:

Physicochemical parameters

Alcohol soluble extractive matter percentage (5.62) as well as water soluble extractive matter percentage (65.06) are indicated the presence of active polor phyto-constituents as Alkaloids, Tannins, Crude fibers, etc. Loss on drying percentage at 105°C temp. (7.41) and ash contained percentage (1.59), ash insoluble percentage (0.10) were found with in permissible as pharmacopeial standard limits which is indicated absence of any mixed foreign adulterated materials and also indicated that the drug is free from any microbial load and aflatoxins contamination in both the analyzed samples. (Table-II).

High Performance Thin Layer Chromatography (HPTLC.) analysis

HPTLC. Studies of alcoholic extractive Majoon-e-Seer Alwi Khani concentrated of both A1, A2 and A3 drug samples showed and indicated the similarity of the drugs. The Rf values of both the Majoon-e-Seer Alwi Khani samples also medicated (Figures 2 and Table-VI.).

Quality control and Quality assurance parameters

Q.C. and Q.A. studies of quality assesment parameters were performed using WHO., IPC.,UPC., (API.UPI.) and AOAC. Standard methods, The microbial load (cfu/gm.) and heavy metals (ppm.) toxic contamination estimation were found within the permissible limits due to control and assessed moisture percentage and ash contained percentage of analyzed parameters which indicated the absence of any adulterative mixed material as well as drugs samples free from contamination (Table- III. & IV.) The Aflatoxins toxic contamination estimation were not detected in the drug samples (Table-V.) drug also Store in cool and dry place in tight closed containers so the drug protected from light and moisture.

CONCLUSION

The Pharmacopeial Standard development, HPTLC. Fingerprinting and Physicochemical research studies of Unani Anti-paralytic drugs Majoon-e-Seer Alwi Khani a classical formulation data have showed that the collected Majoon-e-SeerAlwiKhani drug samples A1, A2 and A3 from Pharmacy of CRIUM., Hyderabad (Under CCRUM., Ministry of AYUSH., Government of India) are authenticated, botanical identical and can be used as the standard Classic Unani formulations. The results of quality control parameters revealed that the drug samples taken for study are free from toxic and hazarded substances like toxic microbes, heavy metals and aflatoxins. Moreover the obtained research studies data and comparative screening will provide the referential supportives information in the development of pharmacopeial standard monographs, identification, reinvestigation, quality assurance and pharmacovigilance of the drug.

ACKNOWLEDGEMENT

I am very thankful to all the supporting scientific & technical staff of DSRU & SMPU. as well as Dr. Munawwar Husain Kazmi, Dy. Director In charge CRIUM., Hyderabad and also grateful thanks to Prof Dr. S. Shakir Jamil, Director General, CCRUM.,(Ministry of AYUSH.), New Delhi, for his valuable guidance, encouragement and providing necessary research facilities.

REFERENCES

1. Hkm. Kabir Uddin, Daftar Almasih. A.D.vBayaz-e-Kabir, (B.K.), Ballimaran, Delhi, 1967; II(II): 189.
2. Anonymous. Physicochemical Standards of Unani Formulations. Central Council for Research in Unani Medicine,5,Panchsheel Shopping Centre,New Delhi-110017, Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Government of India, New Delhi, 1986; I: -95.
3. Anonymous. The Ayuervedic Pharmacopeia of India. The Controller of Publications Civil Lines, Delhi, 1989; I(I): 45-46.
4. Anonymous. Quality Control Methods for Medicinal Plant Materials. World Health Orgnization, Geneva, 1998; 25-28.
5. Anonymous. 2000. Association of Official Analytical Chemists (AOAC).17th Edition.

6. Wagner H, Bladt S. and Zgainski E M .1984. Plant Drug Analysis, A Thin Layer Chromatography Atlas (2nd Edition).Springer-Verlag, Germany.
7. Anonymous. The Ayuervedic Pharmacopeia of India. The Controller of Publications Civil Lines, Delhi, 2006; I(V): 128-130.
8. Anonymous. National Formulary of Unani Medicine, Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Government of India, New Delhi, 2006; I(5): 105-141.
9. Anonymous. The Unani Pharmacopeia of India. Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Government of India, New Delhi, 2007; I(II): 45-46.