

# **RESEARCH ARTICLE**

### DEVELOPMENT OF QUALITY STANDARDS OF BEHIDANA- A PROPHYLAXIS UNANI SINGLE DRUG USED FOR MANAGEMENT OF COVID-19

Sadaf Subhani<sup>1</sup>, R.P.Meena<sup>1</sup>, Asim Ali Khan<sup>1</sup>, S. Mageswasri<sup>2</sup> and P. Meera Devi Sri<sup>2</sup>

1. Central Council for Research in Unani Medicine, M/O Ayush, Govt. of India, New Delhi.

2. Regional Research Institute of Unani Medicine, Royapuram, Chennai, India.

.....

# Manuscript Info

# Abstract

*Manuscript History* Received: 25 May 2020 Final Accepted: 28 June 2020 Published: July 2020

*Key words:-*Behidana, Prophylaxis, Unani single Drug,Pharmacognostical, Physicochemical, HPTLC, Quality Contro The COVID-19 pandemic is threatening the worldwide population. A huge population of world is suffering from this disease and no specific vaccine for this pandemic disease has been developed. The main symptoms of this disease are fever, cough, shortness of breathand tirednessetc. The Ministry of AYUSH has issued various guidelines to prevent this disease by taking immunity boosters and prophylaxis as defence against any disease. In Unani system of medicines, many drugs of plant origin are mentioned in classical literature for strengthening and increasing the immunity of humans. Since, the drug Behidana is being given to COVID-19 patients as prophylaxis regime, it has become necessary to authenticate and develop its pharmacopoeial standards so that quality raw material can be provided to needy mass. The present study is aimed to develop identity, purity and strength of drug using pharmacognostical, physico-chemical and quality control methods.

.....

Copy Right, IJAR, 2020,. All rights reserved.

.....

# **Introduction:-**

The COVID-19 pandemic is a global health crisis at present and is a big threat to the world. It is a pandemic disease of modern time with unique and rapid transmission rate and affected almost all the nations without respecting any border. It's a Novel infection which is caused by SARS CoV-2 with serious clinical manifestation i.e. fever, cough. cold, respiratory distress further may result into death. The government of India has issued various advisories to avoid the spreading of virus infection and AYUSH compositions to boost the immunity. Herbs and herb-derived medicines have played a major role in management of disease for many centuries. Traditionally, a large number of single drugs have been used to treat various ailments in Unani-tibb, and Ayurvedicsystems of medicines. Behidana botanically known as Cydoniaoblonga M. is one of the important Unani single drug used in treatment of various diseases.[1] It belongs to family Rosaceae and isrich in various secondary metabolites such as phenolics, steroids, flavonoids, terpenoids, tannins, sugars, organic acids, and glycosides.[2] The presence of these wide range of phytoconstituents are responsible for pharmacological activities like antioxidant, antibacterial, antifungal, antiinflammatory, hepatoprotective, cardiovascular, antidepressant, antidiarrheal, hypolipidemic, diuretic, and hypoglycemic.[3-5]Almost all parts of Cydoniaoblonga such as seed, buds, bark, leaves, and fruits etc. are used for medicinal purposeas a single drug or as an ingredient in various formulations namely syrup, decoction, confection, semisolid preparations and pill.[6]The Seeds of Behidana contain significant quantities of mucilage and are helpful both in treating bronchitis and as a bulk laxative.[7] They are also used in diarrhoea, dysentery, sore throat and fever[8] Due to it mucilaginous quality it used as a demulcent vehicle for other medicines, specially for skin lotions

both as a remedy for skin conditions and of cosmetic lotions; also used as a stabilizer in dairy preparations.[9] Seed kernel contains glycoside amygdalin, tannin, mucilage (about 22%), ash (1.3%) and fatty oil (14-19%)[10].

In Unani classical literature various properties and actions of Behidana like Dafi'enazla (anti phlegmatic), Dafi'esualhaar (dry cough suppressant), Dafi'ediq (anti tuberculosis), Muzliq (soothing), Mufarrih (exhilarant), Mulattif (demulcent), Musakkin e hararat (heat suppressant), Qabiz (astringent), Mushtaahi (nutrient), Mufattih (de obstruent), Muqavvieme'dda (stomachic), Muqavvi e dil va dimagh (heart and brain tonic) are mentioned [11].In Unani system of medicine, it is used commonly used in treatment of rhinitis, dryness in throat,dry cough, tuberculosis,excess sneezing, fever, dysentery, acidity in the stomach, duodenal ulcer,vomiting, dysuria, haemoptysis, to reduce heat, burning tongue.It is one of the ingredient in someimportant UnaniLaooq e behidana, Banadiq e Buzoor, Sharbateejaz, Jawarish e Safrjal, Habb e Shaqeeqa, Habb e Sil, Habb e Surfaqavi, Qurs e Kaknaj, Laooq e Nazli, Laooq e Sapistan, Laooq e Shamoon.[12]

Keeping in view of its various medicinal uses, the present study was aimed to develop microscopic, powder microscopy, physico-chemical, quality control standards and HPTLC fingerprints of Behidanafor laying down the pharmacopoeial standards which will be helpful to provide quality raw material to COVID-19 patients as immunity booster.

# Materials and Methods:-

#### Pharmacognostical studies:

The seeds of the fruit Cydoniaoblonga Mill. were purchased from the local market, Chennai. The seeds were macroscopically examined for shape, size, surface characteristics, texture, color, odour and taste. The macroscopical, microscopical and powder microscopy were carried out using standard methods [13]. Free hand sections of the seeds were taken and its photographs were taken using digital camera attached with the microscope. [14]

#### **Physioc-chemical parameters:**

Physico-chemical parameters like foreign matter, total ash, acid insoluble ash, loss on drying at 105°C, alcohol and water soluble extractives were carried out as per standard method [15, 16].

# TLC/ HPTLC analysis:

The TLC/HPTLC analysis was performed for chloroform and alcohol extract of the seeds of C. oblonga. [17, 18]

# **Quality control parameters:**

Quality control parameters like microbial load and aflatoxin were carried out as per the WHO guidelines. Heavy metals analysis was done by atomic absorption spectrophotometer. Pesticidal residues were analyzed using GC-MS agilent instrument equipped with mass selective detector as per the methods of AOAC [19, 20].

# **Result and Discussions:-**

# Macroscopic:

Seeds adhere to one another in small irregular masses by dry mucilage, visible in the form of whitish flakes on the surface; seeds obovoid and flat, about 5 to 10mm long, 2 to 5 mm wide and 2mm thick; blackish brown colour; taste slightly bitter; odor not distinct; the two large flattened surfaces meet in a straight acute edge on one side and arched on the surface on the other side, giving the cut surface the appearance of a smooth isosceles triangle.[Fig.1-2]

# Microscopic:

Seed - T. S. of seed ovate in outline with a brown testa consisting of epidermis with numerous mucilage hairs and reddish brown pigment layer of about 7 to 9 rows of rectangular or polygonal cells; endosperm narrow, with polygonal parenchyma separated from pigment layer by a single layer of thin walled parenchyma cells; endosperm cells containing aleurone grains and oil globules; cotyledons present with parenchyma cells filed with aleurone grains and oil globules. [Fig.3-5]

# **Powder Microscopy:**

Cream; epidermis with mucilage hairs; pigment layer; spiral vessels upto 20µ; endosperm cells in surface view with aleurone grains and oil globules and cotyledonary parenchyma cells filled with aleurone grains and oil globules. [Fig.6]

#### **Physico-chemical studies:**

The physico-chemical parameters of the powdered drug were analyzed and the results are shown in **Table -1**. The loss on drying at  $105^{\circ}$ C was found to be 10.95% and the content of total ash and acid insoluble ash found to be 4.34% and 0.86% respectively. The alcohol soluble extractive values 23.60%.

#### Heavy Metal Analysis:

The medicinal plants materials are generally contaminated with arsenic and heavy metals due to environmental pollution. These components even in trace amounts are dangerous and can damage the important human organs such as kidney, liver and heart. The heavy metal contents viz. lead, cadmium, mercury and arsenic were analysed using Atomic Absorption Spectrophotometer and were found within the permissible limits viz. 10, 0.3, 1 and 3 ppm respectively as per WHO guidelines and the results are shown in **Table 2**. Darchini (Stem Bark) is hence considered non-pollutant drug in the environment.

#### Microbial Load Analysis:

The microbial load viz. TBC, TFC, E.Coliand other pathogens were analyzed as per the standard methods and the results are shown in **Table 3**.

#### Analysis of Aflatoxins:

The aflatoxins can be acute toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive to the human being if these are found in the plant material above prescribed limits. The aflatoxins  $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$  were analysed using HPLC and found within permissible limit as shown in **Table 4**. The toxic effect of the drug sample may be considered as nil and hence is safe to use.

#### **Analysis of Pesticide Residues:**

The various pesticidal residues  $\alpha$  - HCH,  $\beta$  - HCH,  $\gamma$  - HCH,  $\delta$  -HCH, op-DDT, pp-DDT, op-DDE,  $\alpha$ - Endosulfan,  $\beta$  – Endosulfan, op-DDD and pp-DDD etc. were tested in the drug sample using GC-MS-MS technique and found within permissible limits. The results are shown in **Table 5.** The drug sample may be considered as pesticide resistant.

# Thin layer Chromatography:

The thin layer chromatographic studies of chloroform and ethanol extracts were carried out using solvent systemstoluene: ethyl acetate: formic acid (8:2:0.4) and toluene: ethyl acetate: formic acid (8:2:0.1) respectively and the results are tabulated in Table 6&7.



 Table 1:- Physico - chemical parameters.

S. No.	Parameters	Results in % (w/w); $n = 3$
1.	Foreign matter	1.33
2.	Loss on drying at 105°C	10.96
3.	Total ash	4.31
4.	Acid insoluble ash	0.86

5.	Alcohol soluble extractive values	23.60
6.	Water soluble extractive values	

# Table 2:- Heavy Metals.

S. No.	Parameter Analyzed	Results	Limits
1	Lead	0.0142 ppm	10 ppm
2	Arsenic	Nil	3 ppm
3	Cadmium	Nil	0.3 ppm
4	Mercury	Nil	1 ppm

# Table 3:- Microbial load.

S. No.	Parameter Analyzed	Results	Limits
1	Total Bacterial Count	2,600 CFU / gm	10 <sup>5</sup> CFU / gm
2	Total Fungal Count	Absent	10 <sup>3</sup> CFU / gm
3	Enterobacteriaceae	Absent	10 <sup>3</sup> CFU / gm
4	Salmonella Spp.	Absent	Nil
5	Staphylococcus aureus	Absent	Nil

# Table 4:- Estimation of Aflatoxins.

S. No.	Aflatoxins	Results	Limits
1	$B_1$	Not detected	0.5 ppm
2	B <sub>2</sub>	Not detected	0.1 ppm
3	G <sub>1</sub>	Not detected	0.5 ppm
4	G <sub>2</sub>	Not detected	0.1 ppm

 Table 5:- Analysis of Pesticide Residues.

S. No.	Pesticide Residues	Results
1	Organo Chlorine Group	ND
2	Organo Phosphorus Group	ND
3	Acephate	ND
4	Chlordane	ND
5	Dimethoate	ND
6	Endosulphan	ND
7	Endosulfan	ND
8	Endosulfon	ND
9	Ethion	ND
10	Endosufon sulphate	ND
11	Fenthion	ND
12	Lindane	ND
13	Methoxychlor	ND
14	Phoratesulfoxide	ND
15	Phoratesulfone ND	
*ND – Not detec	ted	



 $\textbf{Table-6:-} \ \mathsf{R}_f \ \mathsf{Values} \ \mathsf{of} \ \mathsf{Chloroform} \ \mathsf{extract}.$ 

Solvent system	R <sub>f</sub> Values		
	UV 254nm	UV 366nm	VS reagent
Toluene : Ethyl	0.69 Dark green	0.69 Blue	0.72 Grey
acetate : Formic	0.57 Green	0.58 Violet	0.68 Dark grey
acid	0.42 Green	0.35 Grey	0.62 Grey
(8:2:0.4)	0.38 Green	0.28 Blue	0.58 Violet
10µl	0.33 Green	0.18 Blue	0.50 Grey
	0.18 Dark green	0.15 Blue	0.40 Grey
	0.13 Green		0.25 Grey
			0.21 Grey
			0.10 Grey



Fig.8:- HPTLC finger print profile of Behidana - Chloroform extract at 254nm.



Fig. 9:- HPTLC finger print of Behidana – Chloroform extract at 366 nm.



Table-7:- Rf Values of Alcohol extract.

Solvent system	R <sub>f</sub> Values		
	UV 254nm	UV 366nm	VS reagent
Toluene : Ethyl	0.71 Dark green	0.70 Blue	0.73 Grey
acetate : Formic	0.58 Green	0.58 Violet	0.70 Grey
acid	0.35 Green	0.32 Blue	0.62 Grey
(8:2:0.1)	0.23 Green	0.28 Dark blue	0.60 Violet
10µl	0.12 Green	0.26 Grey	0.50 Grey
			0.25 Grey
			0.10 Grey



Fig. 11:- HPTLC finger print of Behidana – Alcohol extract at 254 nm.



Fig. 12:- HPTLC finger print of Behidana – Alcohol extract at 366 nm.

# **Conclusion:-**

To afford a guideline towards authentic identification, this paper describes the morphological and phytochemical characters of a Unani drug obtained from Behidana seeds available on the local market at Chennai. Various chemical and biological tests like phytochemical screening, physicochemical parameters, finger print profile, pharmacognostic features are reported in order to facilitate the identification of the drug respectively. The evaluated microscopic data indicates that the Unani single drug Behidana seeds is authentic. The results of physico-chemical, heavy metals, microbial load, aflatoxins and pesticide residue reveals that the drug is free from toxic substances and hence can be used as prophytactic Unani drug for management of COVID-19 disease.

# Acknowledgement:-

The authors are extremely thankful to the Director General, CCRUM, New Delhi, for providing necessary research facilities

# **References:-**

- 1. Anonymous. Medicinal plants in Folklores of Kashmir Himalayas. New Delhi: CCRUM, Ministry of Health & Family Welfare, Govt. of India. 2001; 96
- 2. Anonymous. Standardization of Single Drugs of Unani Medicine. Part III. New Delhi: Central Council for Research in Unani Medicine (CCRUM). Ministry of Health & Family Welfare, Govt. of India; 1992; 46: 48, 50.
- 3. Sidra Sabir, Rahmatullah Qureshi, Muhammad Arshad, Muhammad ShoaibAmjad, Sammer Fatima, Maria Masood, Saboon, Sunbal Khalil Chaudhari: Pharmacognostic and clinical aspects of Cydoniaoblonga: A review Asian Pacific Journal of Tropical Disease: 2015; 5(11): 850-855
- Muhammad U. Ashraf, Gulzar Muhammad, Muhammad A. Hussain and Syed N. A. Bukhari: Cydoniaoblonga M., A Medicinal Plant Rich in Phytonutrients for Pharmaceuticals; Frontiers in Pharmacology: June 2016; Vol. 7, Article 163.
- SundusKh. Al-Khazraji: Phytochemical screening and antibacterial activity of the crude extract of CydoniaOblonga seeds; Global Advanced Research Journal of Microbiology; Vol. 2(8) pp. 137-140, September, 2013
- 6. Anonymous. The Unani Pharmacopeia of India. Part 1, Vol. II. New Delhi: Ministry of Health and Family Welfare, Govt. of India. 2007; 27-28
- 7. Kabiruddin M. MakhzanulMufradat. New Delhi: IdaraKitabulShifa; 2007; 117, 118.
- 8. IbnSina. Al Qanoon fit Tibb (English Translation) Vol-2. New Delhi: JamiaHamdard; 1998: 261.
- 9. Anonymous. The useful plants of India. New Delhi: National Institute of Science Communication and Information Resources (CSIR); 2006: 154.
- 10. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants. 1st ed. New Delhi: National Institute of Science Communication and Information Resources (CSIR); 2002:86.
- 11. Kabeeruddin M. IlmulAdviaNafeesi. New Delhi: Aijaz Publishing House; 2007:163.
- 12. AyshahFazeenahand Mohamed AleemuddeenQuamri: BEHIDANA (CYDONIA OBLONGA MILLER.) A REVIEW A. H. World Journal of Pharmaceutical Research; 2016 Volume 5, Issue 11, 79-94.
- 13. Johansen D A, 1940. Plant Microtechnique Mc. Graw Hill Book Company Inc. New York and London, 181 186.
- 14. Khare CP. Indian Medicinal plants an illustrated dictionary with 215 pictures of crude herbs. New Delhi: 2007; 190, 191
- 15. Anonymous, 1998. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva. 25 28.
- 16. Quality control of herbal medicine, 2011. World Health Organization.
- 17. Wagner H. And Bladt S. Plant Drug Analysis. A thin layer chromatography Atlas, 2<sup>nd</sup> Edition, Springer Verlag, Germany, 1996; 1-3.
- 18. Sethi P.D. High Performance Thin Layer Chromatography, 1<sup>st</sup> Edition, CBS Publisher and distributers, New Delhi, 1996; 4-28.
- 19. Anonymous, 2005, Official Methods of Analysis of AOAC International, Horwitz W, Latimer G W. (eds). 18<sup>th</sup> Edition. AOAC International: Maryland, Chapter 3, 10-11, Chapter 10; 18-23 and chapter 26; 17.
- 20. Felix D'Mellow, J.P (1997) Handbook of plant anf fungal toxicants, CRC Press Inc, USA; 273-274.