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

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Abstract

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 breath and tiredness etc. 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.

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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 Ayurvedic systems of medicines. Behidana botanically known as Cydonia oblonga M. is one of the important Unani single drug used in treatment of various diseases.[1] It belongs to family Rosaceae and is rich in various secondary metabolites such as phenolics, steroids, flavonoids, terpenoids, tannins, sugars, organic acids, and glycosides.[2] The presence of these wide range of phyto-constituents are responsible for pharmacological activities like antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, cardiovascular, antidepressant, anti-diarrheal, hypolipidemic, diuretic, and hypoglycemic.[3-5] Almost all parts of Cydonia oblonga such as seed, buds, bark, leaves, and fruits etc. are used for medicinal purposes 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

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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’esalhaar (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 some important Unani Laooq e behidana, Banadiq e Buzoor, Sharbateejaz, Jawarish e Safjal, Habb e Shaqeeqa, Habb e Sil, Habb e Surfaqavi, Qurs e Kaknaj, Laoq e Nazli, Laoq e Sapistan, Laoq 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 Behidana for laying down the pharmacopoieal 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 Cydonia oblonga 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]

Physico-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 filled 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°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.Coli and 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 B₁, B₂, G₁ and G₂ 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 α - HCH, β - HCH, γ - HCH, δ -HCH, op-DDT, pp-DDT, op-DDE, α- Endosulfan, β – 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                       |

Behidar - Fruit  
Fig. 1 - Fruit  
Fig. 2 - Seed  
Fig. 3 - T. S. of Seed  
Fig. 4 & 5 - T. S. of Seed (Enlarged)  
Ep – Epidermis  
Pi – Pigmented cells  
En – Endosperm  
Cot - Cotyledons  

Powder  
Fig. 6a – Epidermal cells  
Fig. 6b – Pigmented cells  
Fig. 6c – Endosperm cells  
Fig. 6d – Cotyledonary cells
| S. No. | 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 | 10⁰ CFU / gm |
| 2      | Total Fungal Count            | Absent       | 10⁰ CFU / gm |
| 3      | Enterobacteriaceae            | Absent       | 10⁰ CFU / gm |
| 4      | Salmonella Spp.               | Absent       | Nil         |
| 5      | Staphylococcus aureus         | Absent       | Nil         |

Table 4: Estimation of Aflatoxins.

| S. No. | Aflatoxins | Results  | Limits |
|--------|------------|----------|--------|
| 1      | B₁         | Not detected | 0.5 ppm |
| 2      | B₂         | Not detected | 0.1 ppm |
| 3      | G₁         | Not detected | 0.5 ppm |
| 4      | G₂         | 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 detected
Table-6: R_f Values of Chloroform extract.

| Solvent system | R_f Values          | UV 254nm | UV 366nm | VS reagent |
|----------------|---------------------|----------|----------|------------|
| Toluene : Ethyl acetate : Formic acid (8:2:0.4) 10µl | 0.69 Dark green | 0.69 Blue | 0.72 Grey |
|                | 0.57 Green          | 0.58 Violet | 0.68 Dark grey |
|                | 0.42 Green          | 0.35 Grey | 0.62 Grey |
|                | 0.38 Green          | 0.28 Blue | 0.58 Violet |
|                | 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 |

Solvent System: Toluene : Ethyl acetate : Formic acid (8:2:0.4)

Fig. 7: TLC profile of Chloroform extract
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: R_f Values of Alcohol extract.

| Solvent system                  | R_f Values                  | UV 254nm     | UV 366nm     | VS reagent |
|--------------------------------|-----------------------------|--------------|--------------|------------|
| Toluene : Ethyl acetate : Formic acid (8:2:0.1) 10µl |                             |              |              |            |
|                                 |                             | 0.71 Dark green | 0.70 Blue | 0.73 Grey  |
|                                 |                             | 0.58 Green    | 0.58 Violet | 0.70 Grey  |
|                                 |                             | 0.35 Green    | 0.32 Blue   | 0.62 Grey  |
|                                 |                             | 0.23 Green    | 0.28 Dark blue | 0.60 Violet |
|                                 |                             | 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, fingerprint 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 prophylactic 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

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