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PHYSICOCHEMICAL STANDARDIZATION OF *SUFOOF DAMA HALDI WALA*: A CLASSICAL UNANI FORMULATION

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ABSTRACT

Introduction: *Dama* (Asthma) is one of the leading causes of disease burden when measured in terms of disability adjusted life years and is prevalent in all countries and in all age-groups. There are various formulations mentioned in the Unani classical literature for the treatment of respiratory disorders particularly for *Dama* (Asthma). *Sufoof Dama Haldi Wala* (SDHW) is one such formulation. But, this formulation has not yet been evaluated for its quality standards. Therefore, the present study was carried out to standardize SDHW on the basis of various physico chemical parameters.

Materials and Methods: The ingredients of SDHW are *Triticum aestivum* (Gundum) and *Curcuma longa* (Haldi). The formulation was prepared according to *Bayaze Kabir Vol. II*. The prepared formulation was analysed on the basis of organoleptic parameters; various physico-chemical parameters *viz.* powder characterization, loss on drying, pH, extractive values and ash values; total viable count (TVC) by plate method for microbial load and to check presence or absence of some specific micro-organisms viz. *S. aureus*, *P. aeruginosa*, *E. coli* and *Salmonella sp.*; qualitative analysis of various phytochemical constituents and quantitative estimation of various elements through Atomic Absorption Spectrophotometry (AAS).

Results and Discussion: The results have shown that SDHW was tasteless light yellowish black, shiny fine powder. The quality standards have been developed on the basis of extensive physico-chemical analysis. The microbial load assay and heavy metal analysis were well within normal range and none of the micro-organisms were found in the prepared formulation. The elemental analysis has also shown some interesting leads.

Conclusion: The quality assessment of herbal formulations is of great importance in order to justify their acceptability in contemporary period of medicine. The outcomes of this study may be used as a standard monograph for SDHW and will definitely provide a lead for future pharmacological, pharmaceutical and clinical studies on this formulation.

Keywords: *Sufoof Dama Haldi Wala*, *Dama*, Asthma, Unani Medicine, Standardization.

INTRODUCTION

Dama (Asthma) is prevalent in all countries and in all age-groups but it is the most common chronic disease among children. It is one of the leading causes of disease burden when measured in terms of disability adjusted life years. According to the WHO, more than 339 million people suffered from asthma globally in 2016 and 37·9 million cases were reported in India in the same year [1]. Asthma occurs as a complex interplay between genetic and environmental factors which leads to the swelling and narrowing of airways and may produce extra mucus. This can be a trigger for cough, shortness of breath and wheezing. The disease leads to limitations of daily activities, economic burden of medication and impairments in overall quality of life. A majority (over 80%) of Asthma deaths occur in low and lower-middle income countries [2]. Although appropriate management may control the disease, but there is no cure for Asthma in conventional system of medicine.

On the contrary, there are numerous formulations mentioned in the Unani classical literature for the treatment of respiratory disorders particularly for *Dama* (Asthma) which are effective and inexpensive as well. *Sufoof Dama Haldi Wala* (SDHW) is one of the various Unani formulations used to treat the asthmatic condition and other respiratory diseases since ancient times and find its mention in *Bayaze Kabir* Vol. II [3]. But, this formulation has not yet been evaluated for its quality standards. Standardization of herbal formulations is essential in order to assess their quality, purity and efficacy. The quality assessment of herbal formulations is of great importance in order to justify their acceptability in contemporary period of medicine [4]. Thus, keeping this goal in mind, the present study was carried out to standardize SDHW on the basis of various physico chemical parameters.

Materials and Methods

1. Ingredients of the formulation of SDHW

There are two ingredients in this formulation as mentioned below in table no.1.

Table No. 1: Ingredients of the formulation of SDHW

S. No.	Name of drugs	Scientific Name	Parts Used
1.	Gundum sokhta*	*Triticum aestivum* Linn.	Seeds
2.	Haldi	*Curcuma longa* Linn.	Rhizome

*sokhta means burnt or charred

2. Collection of drugs

The ingredients of SDHW are *Triticum aestivum* (Gundum) and *Curcuma longa* (Haldi) which were purchased from the open market. *Triticum aestivum* (Gundum) was purchased from Khari Baoli, Delhi and *Curcuma longa* (Haldi) was purchased from Shamsi Dawakhana, Ballimaran, Delhi.

3. Identification of drugs

The specimen sample of *Triticum aestivum* (Gundum) was authenticated

by the experts of Department of Ilmul Advia, Ayurvedic and Unani Tibbia College, Karol Bagh New Delhi. The specimen sample of *Curcuma longa* (Haldi) was authenticated by Dr. Sunita Garg, Emeritus Scientist, CSIR-NISCAIR, New Delhi, vide reference number Ref. NISCAIR/RHMD/consult/-2019-3544-45-1. The specimen samples are shown below in figure no. 1.

Triticum aestivum (Gundum) *Curcuma longa* (Haldi)

Figure No. 1: Ingredients of the formulation

4. Preparation of Formulation

4.1 Cleaning and purification of ingredients: Both the drugs were first cleaned, all earthy impurities and foreign matter removed and then dried.

4.2 Preparation of *Gundum Sokhta*: The term *Sokhta* means burnt or charred. In this process the drug is burnt upto the stage when it becomes black or coal like. *Gundum Sokhta* was prepared according to the classical procedure which had been standardized in another study [5].

4.3 Powdering of drugs: Both the ingredients were powdered separately and passed through sieve no. 80.

4.4 Mixing of powder and homogenization: The powder thus obtained was mixed in the ratio 2:1 with two parts of *Gundum Sokhta* and one part of *Haldi* as described in *Bayaze Kabir Vol. II* and was thoroughly mixed in a mixer [3].

5. Standardization of SDHW

5.1 Organoleptic characterization

The prepared formulation was organoleptically characterized on different parameters such as appearance, colour, odour, taste, and texture [6].

5.2 Powder characterization

5.2.1 Bulk Density and Tapped Density: A known quantity (20 gm) of SDHW was carefully put into a measuring cylinder without any losses. The initial volume was noted and the sample was then tapped until no further reduction in volume occurred. This volume was again noted. The initial volume gave the bulk density value and after tapping the final volume gave tapped density value [7].

5.2.2 Hausner's Ratio: Hausner's ratio has been also used as indirect method of quantifying powder flow ability from bulk density [8].

Hausner's ratio = Dt/ Db

Where, Dt = Tapped density and Db = Bulk density.

5.2.3 Car's Index: Car's index has been used as an indirect method of quantifying powder flow ability from bulk density developed by Car. The percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and stability, and is calculated according to following equation [8]:

Car's index (% compressibility) = 100 × (1 - Db / Dt)

Where, Db = Bulk density and Dt = Tapped density

5.2.4 Angle of Repose: The angle of repose gives an indication of the flow ability of the substance. In this method, a funnel was adjusted such that the stem of the funnel lies 2 cm above the horizontal surface. The powdered formulation of SDHW was allowed to flow from the funnel under the gravitational force till the apex of the pile just touched the stem of the funnel, so the height of the pile was taken as 2 cm. A boundary was drawn along the circumference of the pile and diameter was measured by taking the average of six diameters. These values of height and diameter were then substituted in the following equation [8]:

Angle of Repose (θ) = tan -1[2h/d]

Where, h = Height of the pile and d = Diameter of the pile

5.3 Physico-chemical evaluation [9]

5.3.1 Loss on drying at 105°C: An accurately weighed 5gm of SDHW was taken in a petridish. The crude SDHW was heated at 105°C in an oven till a constant weight and percentage moisture content of the sample was calculated.

5.3.2 Determination of pH: The 1% and 10% solution of SDHW was prepared in distilled water (w/v) and pH was determined by using digital pH meter.

5.3.3 Total extractive value: The coarse powder of SDHW was extracted separately in different solvents (water, ethanol, petroleum ether and chloroform) using Soxhlet apparatus. 10 gm powdered SDHW was taken and extracted separately with each solvent. The extracts were filtered using Whatman filter paper (No.1) and evaporated on water bath. Then total extractive values (w/w) were determined in different solvents.

5.3.4 Water soluble extract: Macerated 5gm accurately weighed SDHW with 100 ml of distilled water in a closed flask for 24 hours which was shaken frequently for the first 6 h and then allowed to stand for 18 hours. It was filtered through Whatman filter paper (No. 42) and 25ml of the filtrate was evaporated to dryness in petridish, dried at 105°C, and weighed. The percentage of water soluble extract was calculated with reference to air dried material.

5.3.5 Alcohol soluble extract: Macerated 5gm accurately weighed SDHW with 100 ml of absolute alcohol in a closed flask for 24hours which was shaken frequently for the first 6 h and then allowed to stand for 18 hours. It was filtered through Whatman filter paper (No. 42) and 25ml of the filtrate was evaporated to dryness in petridish, dried at 105°C, and weighed. The percentage of alcohol soluble extract was calculated with reference to air dried material.

5.3.6 Total Ash: Took 5gm of accurately weighed drug was incinerated in a silica crucible previously ignited and weighed. The drug was incinerated in muffle furnace for six hours at 450°C. The ash formed was cooled and weighed. The percentage of total ash was then calculated with reference to air dried material.

5.3.7 Water soluble ash: Boiled the ash for 5mins with 25ml of water, collected the insoluble matter on an ash less Whatman filter paper (No.42), washed with hot water ignited & weighed. The percentage of water soluble ash was then calculated by subtracting the water insoluble ash from total ash.

5.3.8 Acid insoluble ash: Boiled the ash for 5mins with 25ml of 10% v/v HCl, collected the insoluble matter on an ash less Whatman filter paper (No.42), washed with hot water, ignited and weighed. The percentage of acid insoluble ash was then calculated with respect to total ash.

5.4 Qualitative Estimation [10]

The qualitative estimation of SDHW for the presence of following organic constituents was done using different chemical tests.

5.4.1 Test for Alkaloids: It was done by Hager's test. In this, 1 ml of alcoholic extract of the SDHW was taken in a test tube and few drops of Hager's reagent were added. Formation of yellow precipitate will confirm the presence of alkaloids.

5.4.2 Test for Carbohydrates: It was done by Fehling's test. In this, 2ml of aqueous extract of the SDHW was taken in a test tube and 1ml of a mixture of equal parts of Fehling's solution 'A' and 'B' was added and the contents were boiled for 5 minutes. A red or brick red precipitate will confirm the presence of carbohydrates.

5.4.3 Test for Glycosides: Dissolved a small amount of alcoholic extract of the SDHW in 1ml of water, and few drops of aqueous sodium hydroxide solution were added. A yellow colour formation will indicate the presence of glycosides.

5.4.4 Test for Flavonoids: In a test tube containing 0.5 ml of alcoholic extract of the SDHW, 5-10 drops of dilute hydrochloric acid were added followed by a small piece of zinc and the solution was boiled for few minutes. In the presence of flavonoids, a pink, reddish pink or brown colour is produced.

5.4.5 Test for Tannins: In a test tube containing about 5ml of aqueous extract of SDHW, few drops of 1% solution of lead acetate were added. A yellow or red precipitate formation is indicative of tannins.

5.4.6 Test for Phenols: In a test tube 0.5 ml of aqueous extract of SDHW was dissolved in 5ml of distilled water and then 5-8 drops of 1% aqueous solution of lead acetate was added. Formation of yellow precipitate will confirm the presence of phenols.

5.4.7 Test for Saponins: In a test tube containing about 5ml of aqueous extract of SDHW, one drop of sodium bicarbonate solution was added, shaken vigorously and left for 3 minutes. Formation of honey comb like froth will indicate the presence of saponins.

5.5 Quantitative Estimation:

The quantitative analysis of SDHW was done by using sophisticated spectrophotometric and chromatographic techniques.

5.5.1 Detection of heavy metals and estimation of other elements by Atomic Absorption Spectroscopy (AAS): This technique is used to determine the concentration of certain metallic ions in a solution by measuring the intensity of absorption of light at a particular wavelength when a solution of the substance being examined is introduced into a flame.

In this method three standard solutions of the element to be determined were prepared and then introduced each standard solution into the flame three times, and recorded the study reading. A standard calibration curve was plotted by taking the mean of each group of three readings. Then the test solution was introduced into the flame and readings were recorded. This sequence was repeated three times and using the mean of three readings the concentration of the element was determined using the standard calibration curve [11].

5.6 Determination of Microbial load [12]**:** The sample of SDHW was subjected to total viable count (TVC) by plate method and presence or absence of some specific micro-organisms viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella sp*. The pretreated sample was diluted accordingly and 1ml of sample was taken in separate petridishes. Each sample was suspended in appropriate medium like soyabean digest agar medium with 15ml of casein incubated at 30°-35°C for five days to detect bacterial count; and 15ml of sabouraud dextrose agar with antibiotics incubated at 20°-25°C for five days to detect fungal count.

To detect the presence or absence of specific micro-organisms preparation of sample was done as follows:

5.6.1 For *Staphylococcus aureus*: 1ml of sample to 100ml of casein soyabean digest broth was taken, incubated at 35°-37°C for 24-48 hrs, streak on Mannitol salt agar at 35°-37°C for 18-24 hrs.

5.6.2 For *Pseudomonas aeruginosa*: 1ml of sample to 100ml of casein soyabean digest broth was taken, incubated at 35°-37°C for 24-48 hrs, sub-cultured on cetrimide agar, incubated at 35°-37°C for 18-24 hrs.

5.6.3 For *Escherichia coli*: 2ml of sample to 50ml of nutrient broth was taken, incubated at 37°C for 18-24 hrs. 1ml of above sample taken and added 5ml of Mac-conkey broth, incubated at 36°-38°C for 48 hrs. Then sub-cultured on plates of Mac-conkey agar and incubated at 36°-38°C for 24 hrs.

5.6.4 For *Salmonella sp*.: 1ml of sample to 100ml of nutrient broth was taken, incubated at 35°-37°C for 24 hrs. 1ml of above sample taken and added 10ml of selenite F-broth, and then 1ml of above sample taken and added 10ml of tetrathionate-bile brilliant green broth and incubated at 36°-38°C for 48 hrs. Sub-cultured on plates of brilliant green agar and Xylose lysine deoxycholate agar and incubated at 36°-38°C for 18-24 hrs.

Results and Discussion

Firstly *Gundum* was burnt upto the stage of charring till it became coal like known as *Gundum Sokhta* as shown in figure no. 2. Both the drugs were powdered and mixed homogenously in the prescribed ratio. Thus, SDHW was prepared.

Figure No. 2: *Gandum sokhta*

The prepared formulation of SDHW (shown in figure no. 3) was tasteless light yellowish black, shiny fine powder having a typical burnt smell as given in table no. 2.

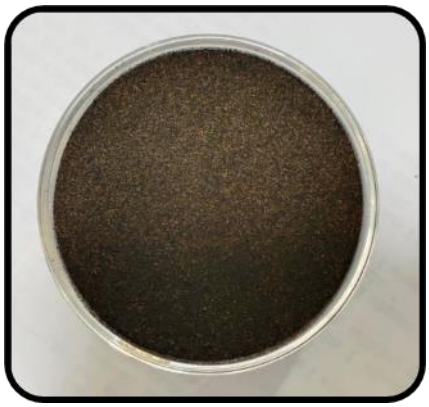

Figure No. 3: *Sufoof Dama Haldi Wala* (SDHW)

Table No. 2: Organoleptic characteristics of SDHW

Parameters	Results
Appearance	Shiny
Colour	Light yellowish Black
Odour	Burnt smell
Taste	Tasteless
Texture	Fine powder

Powder characterization was done on the basis of different parameters and the values are given in Table No. 3.

Table No. 3: Powder characterization of SDHW

Parameters	Percentage mean (n=3) ± SD
Bulk Density (gm/ml)	0.416 ± 0.012
Tapped Density (gm/ml)	0.615±0.002
Carr's index	38.583±0.062
Hausner's Ratio	1.626±0.036
Angle of Repose	24.6°±0.295

The physico-chemical analysis of SDHW was done on various parameters such as moisture content, pH values, extractive values and ash values as described in Table No. 4.

Table No. 4: Physicochemical characterization of SDHW

Parameters	Percentage mean (n=3) ± SD
Loss on drying (mg)	17.266±0.249
pH (1%)	9.40±0.008
pH (10%)	9.50±0.011

Aqueous extract	3.52±0.243
Ethanolic extract	7.88 ±0.495
Petroleum ether extract	2.61±0.029
Chloroform extract	0.696±0.036
Water soluble matter	0.241±0.003
Alcohol soluble matter	0.127±0.006
Total Ash Value (%w/w)	7.0±0.163
Water soluble Ash (% w/w)	0.064±0.003
Acid Insoluble Ash (%w/w)	0.063±0.001

The qualitative analysis of various phytochemical constituents was done and the results showed the presence of alkaloids, glycosides, tannins and phenols. Whereas, carbohydrates, flavonoids and saponins were absent. The results are given below in Table No. 5.

Table No. 5: Qualitative analysis of phytochemical constituents in SDHW

Name of Constituent	**Result**
Alkaloids	Present
Carbohydrates	Absent
Glycosides	Present
Flavonoids	Absent
Tannins	Present
Phenols	Present
Saponins	Absent

Heavy metal detection was done through AAS and it was found that the quantities are well under the permissible limits. Besides these, the formulation was also checked for the presence of some other elements such as aluminium, zinc and selenium. The results are given below in table no. 6.

Table No. 6: Detection of heavy metals and other elements

Name of Element	**Quantity Obtained**
Lead (Pb)	Less than 10 ppm
Arsenic (As)	Less than 3 ppm
Mercury (Hg)	Less than 1 ppm
Cadmium (Cd)	Less than 0.3 ppm
Aluminium (Al)	Less than 0.05 ppm
Zinc (Zn)	Less than 0.3 ppm
Selenium (Se)	0.003 ppm

The prepared formulation of SDHW was checked for microbial load estimation and also analysed for the presence or absence of specific micro-organisms in the formulation. Total bacterial and fungal counts were within the permissible limit and the specific pathogens tested were also absent. The results are shown below in table no. 7 and 8.

Table No. 7: Microbial Load estimation

Test	Result	Normal Range
Total bacterial count	4120 microorganism/gm	Not more than 10^5 microorganisms/gm
Total fungal count	Absent/g	Not more than 10^3 microorganisms/gm

Table No. 8: Test for specific pathogens

S. No.	Name of Pathogen	Result
1	*S. aureus*	Absent
2	*P. aeruginosa*	Absent
3	*E. coli*	Absent
4	*Salmonella sp.*	Absent

Conclusion

The credibility and acceptability of time tested traditional systems of medicines will certainly go up if the quality control parameters for formulations are developed and strictly followed. The test formulation SDHW is one of the various Unani formulations used since ancient times to treat the asthmatic condition and other respiratory diseases. An effort has been made to evaluate this important formulation and establish its quality standards. The assessment was carried out on the basis of various physico-chemical, qualitative and quantitative parameters. The results have shown some interesting outcomes which may be used as a standard monograph for SDHW and will definitely provide a lead for future pharmacological, pharmaceutical and clinical studies on SDHW.

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Conflict of Interest

None to be declared.

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