Physicochemical Standardization of *Habbe Mubarak:* A Unani Compound Formulation

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Abstract

Unani System of Medicine (USM) is based on the drugs originated from plants, animal, and minerals. There is a need to maintain their purity, quality and safety by subjecting the finished products to rigorous scientific testing and to lay down pharmacopoeial standards for both Single and Compound drugs. Standardization of drugs is necessary especially in case of compound formulations to maintain the quality control and to identify the adulterated drugs. So Unani formulation namely *Habbe Mubarak* (HM) was subjected to standardization which is indicated in *Hummae Ajamiya* (malarial fever) and as *Dafe Tap* (antipyretic). The present study was designed to establish the physicochemical standards which can be used for future reference. The physicochemical standardization of HM includes organoleptic characters, weight variation of pill, uniformity in diameter, hardness test, friability test, pH, moisture content, loss of weight on drying, ash values, water and alcohol soluble matter, extractive values, disintegration time, thin layer chromatography (TLC) and total alkaloidal estimation. The findings of physicochemical study of the lab samples of HM may act as reference for quality control.

Keywords: Drug Standardization, Thin Layer Chromatography, *Myrica esculenta, Caesalpinia bonducella*

Introduction

In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is around $ 80 million. But the most important challenges faced by these formulations arise because of their lack of complete standardization. Unani medicines are usually consists of plant origin drugs which are prone to contamination, deterioration and variation in composition. Therefore, quality control of herbal medicines offers a host of problems. To solve this problem, first and foremost task is the selection of the right kind of plant material which is therapeutically efficacious (Panchal *et al.,* 2011).

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process (Kunle *et al.,* 2012).

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This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. Therefore, the study was carried out to evaluate for various physicochemical parameters of the *Habbe Mubarak* (Anonymous, 2006) like ash value, moisture content, pH of 1% and 10% solutions, disintegration time, pill friability, hardness etc. Physicochemical standardization of Unani formulations is essential for the authenticity of the test compounds which provides standard modes for the preparation of the formulations and also describes salient features of identification of the product.

Methodology

To established Physicochemical standards of finished product of Habbe Mubarak (HM) it was prepared according to the formula described in National Formulary of Unani Medicine (NFUM) (Anonymous, 2006) as per WHO guidelines.

Ingredients

1.	Kaifal	*(Myrica esculenta*)	1 part
2.	Maghze Karanjwa	*(Caeslpinia bonducella*)	2 part

Procurement of Raw Drugs

All the ingredients of HM were procured from authorised raw drug dealer in Bangalore and identified by the Unani faculty members supervising the study. Voucher specimens (no. 20/IS/Res./2014) were deposited in the drug museum of NIUM.

Physicochemical Studies

The Physicochemical studies were carried out which included (i) Organoleptic characters of the huboob such as appearance, colour, smell, texture, taste; (ii) Weight variation of pill; (iii) Uniformity in diameter; (iv) Hardness test; (v) Friability test; (vi) pH; (vii) Moisture content; (viii) Loss of weight on drying; (ix) Ash values; (x) Water and alcohol soluble matter; (xi) Extractive values; (xii) Disintegration time; (xiii) Thin layer chromatography (TLC); (xiv) Total alkaloidal estimation.

Organoleptic Properties

Organoleptic evaluation refers to the evaluation of the formulation by colour, odour, taste and texture.The organoleptic characters of the pills were evaluated based on the method of Pandey *et al.* (2012) and Lachman *et al.* (2013).

Weight Variation

Twenty pills were selected randomly and their average weight was determined and pills were also weighed singly. The deviation from the average weight in each case was calculated and expressed as percentage. The pills meet the test, if not more than two pills are outside the limit of 5% (Anonymous, 2006; Lachman *et al.,* 2013).

Uniformity of Diameter

Uniformity of diameter was performed by picking three pills randomly and the diameter was measured individually by Vernier calliper and expressed in mm (Dandagi *et al.,* 2006).

Hardness Test

Three pills were taken and they were individually tested for the hardness by the Monsanto hardness tester in terms of kg/cm (Lachman *et al.,* 2013; William & Wilkins, 2011).

Friability Test

Friability of the pills was determined using Friability test apparatus (Roche's Friabilator) in which combined effect of abrasions and shock in a plastic chamber revolving at 25 rpm and dropping the pill at a height of 6 inches in each revolution. Pre weighed sample of pills was placed in the friabilator and after 100 revolutions collected and de-dusted using a soft muslin cloth and reweighed. The friability (¦) was calculated by the formula

$$\int = \left(1 - \frac{W}{W_0}\right) \times 100$$

Where, W is the weight of the pills before the test and W_o is the weight of the pills after the test (Lachman *et al.,* 2013; Vijaya and Mishra, 2006).

Determination of pH

pH value of 1% and 10% solution: An accurately weighed 1 gm and 10 gm of powder drug was dissolved in accurately measured 100 ml of water separately, filtered and pH measured with a pH meter for 1 % and 10% respectively (Anonymous, 2006).

Moisture Content

The moisture content of the drug was determined by Toluene Distillation method. 10gm of powdered drug was taken in a flask of the apparatus and 75 ml of distilled

toluene added to it. Distillation was carried out for five hours. The volume of water collected in receiver tube was noted and the percentage of moisture calculated with reference to the weight of the air-dried drug taken (Jenkins *et al.,* 2008; Afaq *et al.,* 1994).

Loss of Weight on Drying at 105°C

Two gram of drug was taken, spread uniformly and thinly in a shallow petri dish. It was heated at a regulated temperature of 105ºC, cooled in a desiccator and weighed. The process was repeated many times till two consecutive weights were constant. The percent loss in weight was calculated with respect to initial weight (Anonymous*,* 2006; Afaq *et al.,* 1994).

Ash Values

Total Ash: Two gm of dried powdered drug was incinerated in a silica crucible at a temperature not exceeding 450ºC until free from carbon, cooled and weighed and the percentage was calculated with reference to dried drug.

Acid Insoluble Ash: Total ash was boiled with 25ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on an ash less filter paper washed with hot water and ignited at a temperature not exceeding 450ºC and weighed after cooling. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water Soluble Ash: Total ash was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited. The weight of insoluble ash was subtracted from the weight of the total ash, giving the weight of the water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug (Afaq *et al.,* 1994; Anonymous*,* 2006; Anonymous, 2011).

Determination of Water and Alcohol Soluble Matter

Accurately weighed 4 gm of drug was placed in a glass stoppered conical flask. Macerated with 100 ml of water for 6 hours shaking frequently, and then allowed standing for 18 hours, then shaked well and filtered rapidly through dry filter. 25 ml of the filtrate was transferred to a previously weighed and tarred flat-bottomed dish and evaporated to dryness on a water bath, then dried at 105ºC for 6 hours, cooled and weighed without delay. The percentage of water soluble matter was calculated with reference to the amount of drug taken. The alcohol soluble matter was determined as above by using alcohol in place of water (Anonymous, 2011; Anonymous*,* 2006).

Determination of Extractive values

Successive Extractive Value: The extractive values of pills in different solvents viz. Petroleum ether, alcohol and water were carried out by percolation in soxhlet apparatus. Powdered pills were taken and subjected to successive extraction with each solvent. The heat was applied for six hours on a heating mantle for each solvent. The extracts were filtered using filter paper and after evaporation of the solvents on water bath, the extractive values were determined with reference to the weight of drug (% w/w) (Anonymous, 2006).

Non-Successive Extractive Value: The extractive values of pills in different solvents viz. alcohol and water were carried out separately by percolation in soxhlet apparatus. The heat was applied for six hours on a heating mantle for alcohol and water. Powdered pills were taken and subjected to separate extraction with each solvent (% w/w). The extracts were filtered using filter paper and after evaporation of the solvents on water bath, the extractive values were determined with reference to the weight of drug (Anonymous, 2006).

Disintegration Time

The disintegration time was measured by Disintegration-testing apparatus using DDW as a medium at 37°C. Each of six pills was placed separately in the six cylinders of the two basket rack assemblies of the disintegration apparatus (Lachman *et al.,* 2013; William & Wilkins, 2011).

Alkaloidal Estimation

Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Sutharsingh *et al.,* 2011).

Thin layer Chromatography

Thin layer chromatography was carried out on T.L.C. pre coated aluminium plates, silica gel 60 F 254 (layer thickness 0.25 mm) for alcoholic extract of Habbe Mubarak in benzene: ethyl acetate (3: 1) as mobile phase and for spot detection iodine vapour was used. The R_f values of the spots were calculated by the following formula (Afaq *et al.,* 1994).

$$R_f \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

Results and Discussion

Data of physicochemical evaluation of Habbe Mubarak is based on three readings for each parameter. The organoleptic characteristics i.e. appearance, colour, smell and taste of Habbe Mubarak were found to be spherical round like a pill, brown, non specific and bitter respectively which is the basis for identifying the drug (Table 1, Figure 1).

Table 1: Organoleptic Description of Habbe Mubarak

Appearance	Pill
Colour	Brown
Smell	Non specific
Texture	Hard
Taste	Bitter

Figure 1: Sample of Habbe Mubarak

Weight Variation of Pill: Test is helpful to ensure that a pill contains the proper amount of drug. The % of weight variation of the lab samples was within the prescribed limits of ± 5%. The mean value of randomly selected 20 pills was found to be 504.05± 1.87 mg. (Table 2).

The uniformity of diameter of the circular pills was also measured. The mean value of the diameter of HM was found to be 9.43 ± 0.03 mm. (Table 3).

Table 2: Weight variation of Habbe Mubarak

Sl.No.	Weight of individual Habb (mg)	Absolute difference in weight from mean (mg)	Weight variation (%)
1.	504	0.05	0.01
2.	489	15.05	2.98
3.	496	8.05	1.59
4.	510	5.95	1.18
5.	499	5.05	1.00
6.	497	7.05	1.39
7.	499	5.05	1.00
8.	493	11.05	2.19
9.	509	4.95	0.98
10.	494	10.05	1.99
11.	517	12.95	2.57
12.	503	1.05	0.21
13.	501	3.05	0.61
14.	505	0.95	0.19
15.	510	5.95	1.18
16.	508	3.95	0.78
17.	520	15.95	3.16
18.	504	0.05	0.01
19.	517	12.95	2.57
20.	506	1.95	0.39
Mean ± SEM	504.05 ± 1.87		

Hardness test is done to determine the force required to break the sample along its diameter. The mean value of the hardness of HM was found to be 4.4 ± 0.12 kg/cm (Table 3).

Friability test is done to determine the possible reduction in the weight of the solid dosage forms as a result of the mechanical erosion during handling and transportation. The mean percentage of friability of HM was found to be 0.19 ± 0.01 % (Table 3).

pH value of the drug is also an important parameter because weak acids would be better absorbed from the stomach than from the upper intestine. pH of HM was found to be slightly acidic for the drug and the value were found to be 6.30 ± 0.01 and 5.92 ± 0.01 in 1% and 10% aqueous solution respectively (Table 4).

Moisture content for detecting the quality of the drugs; excessive moisture content affects the quality of the drug and also its efficacy and more moisture becomes ideal medium for the growth of the bacteria and fungi which spoil the quality of the drug. The percentage of moisture content in HM was found to be 5.33 ± 0.33 (Table 4).

Table 3: Diameter, Hardness and Friability of Habbe Mubarak

Sl.No.	Diameter of Pill (mm)	Hardness (kg/cm)	Friability (%)
1.	9.5	4.6	0.20
2.	9.4	4.4	0.20
3.	9.4	4.2	0.17
Mean ± SEM	9.43 ± 0.03	4.4 ± 0.12	0.19 ± 0.01

Table 4: pH Values, Moisture content by Toluene Distillation Method and Loss of Weight on drying of Habbe Mubarak

Sl.No.	pH Values		Moisture Content (%)	Loss of weight on drying (%)
	1% Solution	10% Solution		
1.	6.32	5.92	5	5.15
2.	6.28	5.94	5	5.76
3.	4.30	5.90	6	6.14
Mean±SEM	6.30 ± 0.01	5.92 ± 0.01	5.33 ± 0.33	5.68 ± 0.29

Loss of weight on drying is done to determine the amount of water, volatile matter or mass in the sample. The percentage loss of weight on drying was found to be 5.68 ± 0.29 (Table 4).

Ash value of the drug is done for the detection of impurities and adulteration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it but it may also gives information related inorganic matter added for the purpose of adulteration of the drug. The mean percentage values of the total ash, acid insoluble ash and water soluble ash were found to be 11.95 ± 0.03 %, 0.43 ± 0.04 % and 3.21 ± 0.29 % respectively (Table 5).

Water and Alcohol soluble matter: The amount of extract that a drug yields to a given solvent is often an approximate measure and act as an index for some drugs. The mean percentages of alcohol and water soluble matter were found to be 12.93 ± 0.13 and 9.07 ± 0.08 respectively (Table 6).

Extractive values helps in the determination of the adulteration and is an index of the purity of the drug. The mean percentages of the non-successive extractive values were found to be 24.81 ± 1.28 and 14.88 ± 0.11 with water and alcohol, respectively and successive extractive values were found to be 11.77 ± 0.03, 5.62 ± 0.06 and 16.28 ± 0.03 in petroleum ether, alcohol and water, respectively (Table 7).

Disintegration test is done to determine whether tablets, capsules and pills disintegrate within the prescribed time and breaks down into smaller particles.

Table 5: Ash Value of Habbe Mubarak

Sl.No.	Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)
1.	11.98	0.49	3.78
2.	11.99	0.44	2.89
3.	11.89	0.34	2.95
Mean ± SEM	11.95 ± 0.03	0.43 ± 0.04	3.21 ± 0.29

Table 6: Alcohol and Water soluble matter of Habbe Mubarak

Sl.No.	Alcohol soluble matter (%)	Water soluble matter (%)
1.	13.15	9.23
2.	12.70	9.03
3.	12.93	8.95

The mean value of disintegration time in aqueous medium was found to be 29.33 ± 0.88 minutes (Table 8).

Total Alkaloids: As a medicinal agent, alkaloids are characterized by their high potency. The mean value of total alkaloidal estimation of HM was found to be 0.15 ± 0.02 % (Table 8).

Thin layer chromatography is an important parameter used for detecting the adulteration for analysing the quality and purity of the drugs. Six spots were found on TLC silica plate with the alcoholic extract of HM. The R_f values were found to be 0.014, 0.271, 0.518, 0.612, 0.776, and 0.906 (Table 9, Figure 2).

Table 7: Non-Successive and Successive Extractive Values of Habbe Mubarak

Sl.No.	Non-Successive Extractive Value		Successive Extractive Values		
	Water (%)	Alcohol (%)	Petroleum ether (%)	Alcohol (%)	Water (%)
1.	23.91	15.07	11.75	5.63	16.29
2.	27.34	14.89	11.82	5.51	16.22
3.	23.18	14.68	11.73	5.72	16.34
Mean ± SEM	24.81 ± 1.28	14.88 ± 0.11	11.77 ± 0.03	5.62 ± 0.06	16.28 ± 0.03

Table 8: Disintegration time and Total Alkaloidal Estimation of Habbe Mubarak

Sl.No.	Disintegration time in Aqueous medium (min)	Total Alkaloidal Content (%)
1.	29	0.18
2.	28	0.12
3.	31	0.16
Mean ± SEM	29.33 ± 0.88	0.15 ± 0.02

Table 9: TLC of Habbe Mubarak

Extract	Solvent	Treatment	No. of Spots	R_f Value	Colour
Ethanol	Benzene: Ethyl acetate (3: 1)	Iodine Vapour	6	0.014, 0.271, 0.518, 0.612, 0.776 and 0.906	Yellow

Figure 2: TLC of Habbe Mubarak

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