Physico-chemical Standardization of *Habbe Kafoori:* A Unani Formulation

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Abstract

Habbe Kafoori (HK pills) is a pharmacopoeal compound Unani preparation mainly used in patients of pyrexia of different etiology. Many pharmaceutical companies prepare it for commercial supply but they frequently fail to maintain the desired standards of its quality. In the present study HK has been studied on a number of physicochemical parameters to set its physicochemical standard. The parameters include organoleptic characters, weight variation of pills, uniformity in diameter, hardness test, friability test, pH, moisture content, loss of weight on drying, ash values, water and alcohol soluble matter, extractive values, Thin Layer Chromatography (TLC) and total alkaloid estimation. The findings of the study may be used as a reference for future studies and also to test the drugs available in the market for their quality and expected biological activity.

Keywords: Organoleptic, Thin Layer Chromatography, Alkaloidal estimation.

Introduction

Habbe Kafoori (HK) is an important drug of Unani system of medicine commonly used in the management of *Hummiyate Moharraqa, Humma Diqe Mewi* and other fevers of diverse etiology (Anonymous, 2006). It is a pharmacopoeal drug (pills) which is prepared by physicians and pharmacists and also by pharmaceutical units for large scale supply. Since the agents intended to be used for therapeutic purposes are studied for their safety and efficacy in order to ensure their quality and expected pharmacological and therapeutic effect therefore, it is mandatory to test such agents on physicochemical parameters to set their standards of quality. Standardization is considered essential for herbal drugs and their formulations in order to assess their quality. Since there are chances of variation in different batches of medicine it is imperative to establish a system of standardization for every plant product in the market.

If the physico-chemical standards are ensured then there are greater chances that the drug is effective therapeutically and its different samples will produce uniform degree of effect. Since *Habbe Kafoori* has been not been studied thoroughly for its physicochemical standards therefore in the present study it was prepared according to the methods described in Unani pharmacopoeal literature and studied on various physicochemical parameters including ash value, moisture content, pH of 1% and 10% solutions, pill friability, hardness and TLC etc. The findings will help in determining the quality of the test drug available in the market and set the standard for a genuine preparation.

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Materials and Methods

Unani poly herbal formulation *Habbe Kafoori* (Anonymous, 2006) was prepared according to the method described in national formulary. It was then studied on certain physicochemical parameters.

Ingredients

Kafoor (*Cinnamomum camphora*) 3 g, Tabasheer (*Bambusa arundinacea*) 5 g, Nishasta (*Triticum sativum*) 5 g, Sandal Safaid (*Santalum album*) 5 g, Maghze Tukhme Kadu (*Lagenaria siceraria*) 5 g, Kateera (*Cochlospermum gossypium*) 5 g, Luabe Behidana (*Cydonia oblonga*) Q.S

Procurement of crude drugs

The ingredients of HK were procured from an authorized single drug dealer in Bangalore. Unani experts including the supervisors of the study at National Institute of Unani Medicine (NIUM), Bangalore identified the crude drug samples. A Voucher specimen (No. 21/IS/Res./2014) has been deposited in the museum of NIUM.

Physicochemical studies

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

Organoleptic properties

Organoleptic evaluation refers to the evaluation of the formulation by colour, odour, taste and texture. These were evaluated according to the method recommended by Pandey *et al*. (2012).

Weight variation

Average weight of twenty randomly selected pills was determined then each pill was weighed singly. In each case the deviation from the average weight was calculated and expressed as percentage. The pills are considered within the range if not more than two pills are outside the limit of 5% (Anonymous, 2006; Lachman *et al*., 2013).

Uniformity of diameter

Three pills were picked randomly to measure uniformity of diameter individually by Vernier calliper and expressed in mm (Dandagi *et al*., 2006).

Hardness Test

Three pills were individually taken and 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). Pre weighed sample of pills was placed in the friabilator and was subjected to 100 revolutions. Pills were de-dusted using a soft muslin cloth and reweighed. The friability (*f*) was calculated by the formula

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

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).

Determination of pH

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

Moisture Content

Toluene distillation method was used for the determination of moisture content of the drug. 10 gm of powdered drug was taken in a flask and 75 ml of distilled toluene was 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 was calculated with reference to the weight of the air-dried drug (Jenkins *et al*., 2008; Afaq *et al*., 1994).

Loss of weight on drying

Two gram of drug was taken in a Petri dish and was spread uniformly. It was heated at a temperature of 105ºC, cooled and weighed. The process was repeated 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 450 ºC until free from carbon; cooled and weighed and the percentage was calculated with reference to air dried drug.

Acid insoluble Ash: Total ash was boiled with 25 ml of dilute hydrochloric acid for 5 minutes and insoluble matter was collected on an ash less filter paper washed with hot water and ignited at a temperature not exceeding 450 ºC 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

Four gm of accurately weighed drug was placed in a glass stoppered conical flask. It was macerated with 100 ml of water and alcohol separately for 6 hours and was shook frequently, and then allowed standing for 18 hours, and filtered rapidly through a 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 105 ºC for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The percentage of water or alcohol soluble matter was calculated with reference to the amount of drug taken (Anonymous, 2011).

Determination of extractive values

Successive extractive value: Powdered pills were taken and subjected to successive extraction with different solvents viz. petroleum ether, alcohol and water, respectively. It was carried out by percolation in Soxhlet apparatus. 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: Powdered pills were taken and subjected to separate extraction with each solvent (% w/w) viz. alcohol and water and was carried out separately by percolation in Soxhlet apparatus. The heat was applied for six hours. 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).

Alkaloidal estimation

Five gram of drug sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to it and allowed to stand for 4 hours. It 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 process of precipitation completed. The whole solution was allowed to settle; the precipitate was collected and washed with dilute ammonium

hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Suthersingh *et al*., 2011).

Thin layer Chromatography

Thin layer chromatography was carried out on TLC pre coated aluminium plates with silica gel 60 F 254 (layer thickness 0.25 mm) for alcoholic extract of HK in benzene: ethyl acetate (3:1) as mobile phase. For spot detection iodine vapour was used. The R_f values of the spots were calculated by the following formula (Jenkins *et al*., 2008).

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

Table 1: Organoleptic description of Habbe Kafoori

Appearance	Pill
Colour	Straw
Smell	Like Kafoor and Sandal
Texture	Hard
Taste	Bitter and acrid

Table 2: Weight variation of Habbe Kafoori

Sl. No	**Weight of individual Habb (mg)**	**Difference in weight from mean(mg)**	**Weight variation** (%)
1.	507	2.15	0.43
2.	493	11.85	2.35
3.	505	0.15	0.03
4.	512	7.15	1.42
5.	499	5.85	1.16
6.	517	12.15	2.40
7.	489	15.85	3.14
8.	502	2.85	0.56
9.	516	11.15	2.21
10.	497	7.85	1.55
11.	506	1.15	0.23
12.	512	7.15	1.42
13.	508	3.15	0.62
14.	493	11.85	2.35
15.	510	5.15	1.02

Sl. No	Weight of individual Habb (mg)	Difference in weight from mean(mg)	Weight variation (%)
16.	513	8.15	1.61
17.	505	0.15	0.03
18.	499	5.85	1.16
19.	513	8.15	1.61
20.	501	3.85	0.76
Mean ± SEM	504.85 ± 1.80		

Table 3: Diameter, Hardness and Friability of Habbe Kafoori

Sl. No	Diameter of Pill (mm)	Hardness (kg/cm)	Friability (%)
1.	9.8	6.4	0.07
2.	10	6.7	0.10
3.	10.1	6.6	0.10
Mean ± SEM	9.97 ± 0.09	6.57 ± 0.09	0.09 ± 0.01

Table 4: pH Values, Moisture content and Loss of Weight on drying of Habbe Kafoori

Sl. No	pH Values		Moisture Content (%)	Loss of weight on drying (%)
	1% Solution	10% Solution		
1.	6.03	5.80	5	7.95
2.	6.02	5.78	6	7.84
3.	6.04	5.82	6	9.23
Mean ± SEM	6.03 ± 0.01	5.8 ± 0.01	5.67 ± 0.33	8.34 ± 0.45

Table 5: Ash Values of Habbe Kafoori

Sl. No	Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)
1.	18.08	15.11	3.29
2.	18.06	15.22	1.25
3.	17.99	15.06	1.49
Mean ± SEM	18.04 ± 0.03	15.13 ± 0.05	2.01 ± 0.64

Table 6: Alcohol and Water soluble matter of Habbe Kafoori

Sl. No	Alcohol soluble matter (%)	Water soluble matter (%)
1.	5.98	3.03
2.	5.75	2.78
3.	5.53	3.30
Mean ± SEM	5.75 ± 0.13	3.03 ± 0.15

Table 7: Non-successive and successive extractive values of Habbe Kafoori

Sl. No	Non-Successive Extractive Values		Successive Extractive Values		
	Water (%)	Alcohol (%)	Petroleum ether (%)	Alcohol (%)	Water (%)
1.	3.89	10.23	7.03	2.69	2.45
2.	4.72	9.52	7.05	2.72	2.48
3.	5.83	8.98	7.10	2.66	2.72
Mean ± SEM	4.81 ± 0.56	9.58 ± 0.36	7.06 ± 0.02	2.69 ± 0.02	2.55 ± 0.08

Table 8: Total Alkaloidal Estimation of Habbe Kafoori

Sl. No	Total Alkaloidal Content (%)
1.	0.34
2.	0.3
3.	0.28
Mean ± SEM	0.307 ± 0.02

Table 9: TLC of Habbe Kafoori

Extract	Solvent	Treatment	No. of Spots	Rf Value	Colour
Ethanol	Benzene:Ethyl acetate (3:1)	Iodine Vapour	4	0.14, 0.705, 0.769, and 0.846	Yellow

Figure 1: Sample of Habbe Kafoori

Figure 2: TLC of Habbe Kafoori

Results and Discussion

Physico-chemical studies

The organoleptic characteristics i.e. appearance, colour, smell and taste of HK were found to be round in shape, straw coloured, having Kafoor and Sandal like smell and bitter and acrid in taste (Table 1) (Figure 1).

Weight variation of pill: The mean value of randomly selected 20 pills was found to be 504.85±1.80 mg. This test was done to help ensure that a pill contains the proper amount of drug. All 20 pills when weighed individually were found to be within the permissible limit of 5 % (Table 2).

Diameter: Diameter was measured to ensure the uniformity in size of the pills and the amount of drug. The mean value of the diameter was found to be 9.97 ± 0.09 mm (Table 3).

Hardness: The mean value of the hardness was found to be 6.57 ± 0.09 kg/cm, this test was done to determine the force needed to fracture or break the formulation (Table 3).

Friability: The mean percentage of friability was found to be 0.09 ± 0.01. Test was done to find out any possible reduction in the weight of the solid dosage forms as a result of mechanical erosion during handling, packaging and transportation due to removal of the small fragments and particles from the surface of the solid dosage forms (Table 3).

pH Values: pH value of the drug was determined in 1% and 10% aqueous solution and the values were found to be 6.03 ± 0.01 and 5.8 ± 0.01, respectively (Table 4).

Moisture content: It is a good parameter for detecting the quality of the drugs. Variation in moisture level affects the quality of the drug and also its efficacy. The mean percentage of the moisture content was found to be 5.67 ± 0.33 (Table 4).

Loss of weight on drying: It is undertaken to determine the amount of water or volatile matter in the sample, which is removed during drying process. The mean percentage of loss of weight on drying was found to be 8.34 ± 0.45 (Table 4).

Ash value of the drug was determined for detecting inorganic matter of the drug. An ash determination is a basis of judging the identity of the drug and gives information related to its adulteration with inorganic matter. The mean percentage values of the total ash, acid insoluble ash and water soluble ash were found to be 18.04 ± 0.03, 15.13 ± 0.05 and 2.01 ± 0.64, respectively (Table 5).

Alcohol and water soluble matter: Amount of drug soluble in a given solvent is an index of its purity. The mean percentage of alcohol and water soluble content was found to be 5.75 ± 0.13 and 3.03 ± 0.15, respectively (Table 6).

Extractive value is an index of the purity of the drugs and helps in the determination of adulteration and variation if any, in the chemical constituents because it leads to the change in the extractive values. The mean percentage of the non-successive extractive values in water and alcohol were found to be 4.81 ± 0.56 and 9.58 ± 0.36, respectively. The mean percentage of the successive extractive values with petroleum ether, alcohol and water were found to be 7.06 ± 0.02, 2.69 ± 0.02 and 2.55 ± 0.08, respectively (Table 7).

Alkaloids are characterized by their high potency and efficacy. Little variation in alkaloid may cause a major change in the efficacy of drug. The mean value of total alkaloidal estimation of HK was found to be 0.307 ± 0.02 % (Table 8).

Thin layer chromatography: It is one of the important parameters used for judging the quality of the drugs and for detecting the adulteration. Four spots were found on TLC silica plate with the alcoholic extract of HK. The Rf values were found to be 0.14, 0.705, 0.769, and 0.846 (Table 9, Figure 2).

The findings of the study sets the physicochemical standards of HK which may be used for future reference and may be used to compare the test drugs intended to be used in the management of diseases. Since the biological activity of plant drugs and their preparations depends mainly on the authenticity of the ingredients and the procedure used for their processing and final preparation therefore physicochemical parameters are considered important to ensure the quality and thereby efficacy of a drug. Since HK is a commonly used drug and is prepared by many pharmaceutical companies therefore the findings of present study will help the practitioners and pharmaceutical industries alike.

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