Standardiztion of Unani Ointments: 'Marham Kafoor'

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Abstract

Marahim (Ointments) are the important formulations of Unani System of Treatments, used as topical applicant for cuts, pains and abrasions etc. Most of the ointments contain mineral and/or plant products that vary from formulation to formulation. No work on standardization of 'Marahim' (Ointments) has been done till date, therefore, a series of the work on different ointments of Unani System of Medicine have been started and in the present paper the works on standardization and quality assurance of an ointment (Marhaam kafoor) are reported. The parameters that are selected are those that are recommended by National Unani Pharmacopoea Committee. 'Marham Kafoor' is a white, semisolid compound with camphorous smell. Its action in mentioned as 'Mubarrid' and 'Daf-e-Taffun', in Unani literature and the mode of administration is topical (Anonymous, 1971; Anonymous, 2008). The parameters that are studied are Total ash (8.33%), Acid insoluble ash (0.82%), Water soluble ash (0.15%), Alcohol soluble matter (5.84%), Water soluble matter (1.00%), Pet. ether soluble matter (36.23 %), Water content (4.22%), Loss on drying (5.5%), Total Zinc as Zinc oxide (0.8-0.9%) and Congealing point (62 - 65^{0}C). Thin Layer Chromatography (TLC) profile are also used for finalizing the marker compounds. The heavy metals, aflotoxins and pesticidal residue are not detected. No microbs were noted in the final product. In addition HPLC profile of 'Marham Kafoor' are also recorded for future reference.

Keywords: Marham Kafoor; Standardization; Quality control; Ointment

Introduction

With the increase of global interest in traditional system of medicine, issue of quality, efficacy and safety of Ayurvedic, Siddha and Unani drugs has attained the renewed attention of scientists, and there is need of sufficient scintific data in order to enforce acceptance of these traditional medicines in masses of India and other countries. Marahim (Ointments) are the important preparation of Unani Medicine, used as topical applicant for cuts, pains and abrasions *etc*. Most of the ointments contain mineral and/or plant products that vary from formulation to formulation. No work on standardization of such type of drugs has been done till date, therefore, a series of work has been initiated to standardize the ointments for maintaining the quality and efficacy. For the present study 'Marham Kafoor' is selected and standardization is made. The work on others ointments will be reported else where. The parameters that are selected are those which are recommended by National Unani Pharmacopoea Committee.

Materials and Methods

Raw Materails:

The formulation contains the ingridients (Table 1) that are mentioned in part Vth of National Formulary of Unani Medicine (Anonymous, 2008). The raw materials were purchased from the market and their identity, purity and strength were checked as per reference, given in table 1.

Preparation of Ointment

In an open mouth bottle the egg albumin and methyl alcohol were added and the content stirred till egg albumin dissolved in alcohol. After filtration with muslin cloth the content was preserve for further processing. In another bottle small pieces of recommended quantity of camphor were dissolved in methyl alcohol and preserved for further processing. In a stain less steel pan, oil of 'Kunjud' (*Sesamum indicum* Linn.) was boiled and in hot oil, the desired quantity of bees wax was added and kept on gas burner till wax was completely dissolved. The pan was removed from the burner and Zinc oxide (Safeda Kashgari) was added and stirred till it takes the consistency of an ointment. The egg albumin and camphor which was dissolved in methyl alcohol was mixed and stirred to get the homogenous mixture.

Physicochemical Parameters

Physicochemical studies like total ash; acid insoluble ash; water soluble ash; alcohol, petrolium Ether and water soluble matter; water content; loss on drying; total zinc as oxide and congealing point were determined quantitatively according to methods recoeded in Indian Pharmacopoeia, WHO guidelines and methods mentioned by Afaq *et al* (Anonymous, 1978(a); Anonymous, 1978(b); Anonymous, 2005 and Afaq *et al*, 1994). Thin Layer Chromatography was conducted (Harborne, 1973). The HPLC for determination of pesticidal residue and atomic absorption for heavy metals determination was used. The presence of aflotoxins and microbial load were studied as per revised recomendation of WHO mentioned in its bulletin (Anonymous, 2005).

Estimation of Zinc oxide

The ointment was ignited in a porcelain crucible till free of carbon. 1.5 g material was dissolve and 2.5 g of ammonium chloride in 50 ml of 1N sulphuric acid. Excess of acidic solution was titrated with I N sodium hydroxide using solution of methyl orange as indicator. Each ml of I N Sulphuric acid is considered as equivalent to 0.04069 mg ZnO (Anonymous, 1978(a))

HPLC analysis

Common pesticide (Chloropyriphos, DDT, Parathion, Malathion and Endosulphan) were obtained from Sigma-Aldrich and dissolved in acitonitrile. This standard was injected in the C18 column (30 cm) fitted in the HPLC instrument (Cyber lab, USA) and software driven peaks obtained. The pressure was 6.5 Pa and temperature was 250C.The Flow rate was 1.0 ml/min. The detector was UV and the wavelength was 254 nm. The mobile phased was acitonitrile: water (75:25). The drug samples were also injected and the peaks appears were compared with the peaks of pesticides (Fig. 1), considering the retention time in the same conditions. The general HPLC profile of drug were also recorded and given in figure (Fig.2).

Results and Discussion

The present study is an attempt to ascertain the pharmacopoeial standards for the standardization of 'Marham Kafoor'. Total ash (8.33%), Acid insoluble ash (0.82%), Water soluble ash (0.15%), Alcohol soluble matter (5.84%), Water soluble matter (1.00%), Pet. ether soluble matter (36.23 %), Water content (4.22%), Loss on drying (5.5%), Total Zinc oxide (0.8-0.9%) and Congealing point (62 - 65^0C), are the parameters considered as tools of checking the quality, identity, purity and strength of the ointment. The HPLC profile of the drug was recorded as the obtained graph can be compared with the batches in future. The HPLC pattern shows 29 peaks and peak number 9 is the major peak. The percentage composition of this compound is 86.154%. This peak is followed by peak number 6 (3.217%) and peak number 5 (2.327%). The total percentage composition of these three compounds is 91.698%. Peak number 7,8,10 and 14 are comparatively smaller peak and the percentage compositions of compounds are 1.817%, 1.867%, 1.406% and 1.139% respectively. Other peaks show non significant concentration, so for checking the quality of future batch peak number 5, 6 and 9 should be compared. The change in the profile of any batch will be an indication for low quality or adulteration. Thin Layer Chromatography (TLC) profile (Table 4) and Rf value obtained alongwith photographs of the TLC plate (Fig. 3) was also recorded for future refernce. The heavy metals, aflotoxins, pesticidal residue and microbial load were also studied and reported (Table 3a, 3b, 3c, 3d) No growth of any Fungi or Bacteria were observed in the cultural media and no aflotoxines (B1,B2,G1,G2) were detected. Pb, Hg, and As are detected in the formulation but present with in the limit. The HPLC analysis shows no commonly used pesticide as in HPLC profile of drug and there is no any peak correspond to peak number 2,4,5,7, and 8 of soft ware driven HPLC graph of the mixture of

different pesticides on the same instrument and under the same conditions (Fig.1; Table 5). The presence of heavy metals is due to the presence of these metals in the Zinc oxide used for prepration of ointment but all are with in limits. No aflotoxin, and microb were detected, hence passing all the test for its clinical use. As a topical applicant it is safe and reported effective for minor infections. This semisolid white ointment has campharous odour and during preperation of one batch 5% loss is expected.

Table 1: Ingredients of Marham Kafoor

S. No.	Unani Name	Botanical/ English Name	Part Used	Reference	Quantity
1	Safeda Kashgari	Zinc Oxide	Zinc Oxide	IP; 1978, pp 550*	60 g
2	Kafoor	Cinnamomum camphora Linn.	Crystals from oil	IP, 1978, p 99*	15 g
3	Roghaan kunjad	Sesamum indicum Linn.	Oil of seed	IP, 1978, p. 442*	450 ml
4	Mom Asli	Beeswax	Wax from honey comb	IP, 1978, p. 62*	150 g
5	Alcohol Khabshi	Methyl Alcohol	Methyl Alcohol	IP, 1978, p. 589*	20 ml
6	Egg	Egg albumin	White albumin	—	5 pieces

*IP=Indian Pharmacopoeia

Table 2: Physicochemical Properties of Marham Kafoor

Parameter*	Marham Kafoor
Total ash	Not more than 8.33%
Acid insoluble ash	Not more than 0.82%
Water soluble ash	Not more than 0.15%
Alcohol soluble matter	Not less than 5.84%
Water soluble matter	Not less than 1.00%
Pet. ether soluble matter	Not less than 36.23 %
Water Content	Not more than 4.22%
Loss on dry	Not more than 5.5%
Total Zinc oxide	0.8-0.9%
Congealing point	62 - 65 ^{0}C

***Each parameter is mean of three experiments**

Table 3: Heavy Metals (a), Microbial Load (b), Aflatoxin (c) and Pesticide residue (d) of 'Marham Kafoor'

(a) Qualitative Analysis for Heavy Metals

S. No.	Test Parameters	Results*	Limits
1	Lead as Pb	1.818 ppm	Not more than 10 ppm
2	Mercury as Hg	0.726 ppm	Not more than 10 ppm
3	Arsenic as As	0.134 ppm	Not more than 3.0 ppm
4	Cadmium as Cd	Not Detected	Not more than 0.3 ppm

(b) Microbial Load (for three samples)

S. No.	Microbs	Result*	Limit
1	Total Bacterial Count	Nil	Not more than 105 /g
2	Total Fungal Count	Nil	Not more than 103/g
3	*Enterobacteriaceae*	Nil	Nil
4	*Salmonella*	Nil	Nil
5	*Staphylococcus aureus*	Nil	Nil

(c) Aflatoxin (for three samples)

S. No.	Aflatoxin	Result*	Limit
1	B1	Not detected	Not more than 0.50 ppm
2	B2	Not Detected	Not more than 0.10 ppm
3	G1	Not Detected	Not more than 0.15 ppm
4	G2	Not Detected	Not more than 0.10 ppm

(d) Pesticide residue (for three samples)

S. No.	Pesticide	Result*	Limit
1	Chloropyriphos	Not detected	Not more than 0.2 mg/kg
2	DDT	Not detected	Not more than 1.0 mg/kg
3	Endosulphan	Not detected	Not more than 3.0 mg/kg
4	Malathion:	Not detected	Not more than 1.0 mg/kg
5	Parathion	Not detected	Not more than 0.5 mg/kg

Note. *All result based on three experiments

Table 4: Thin Layer Chromatography Profile of Marham Kafoor

Drugs	Extract	Mobile Phase	Spraying Reagent	Observation
Marham Kafoor	Methanolic	Chloroform: Toluene: Ethyl acetate (1:1:1)	Vanillin H2SO4 Iodine vapors	After spray of Vanalne Sulphuric acid seven spots appears; Rf.10, 0.20, 0.30, 0.40, 0.45, 0.56, 0.90 After exposure in Iodine Vapour Six spots appears; Rf. 0.10, 0.20, 0.30, 0.4, 0.60, 0.90

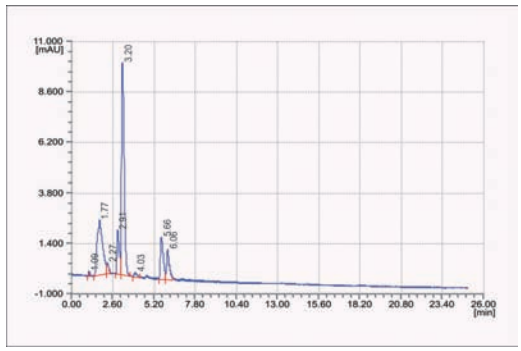

Fig. 1. HPLC of the Mixture of different pesticides

Table 5: HPLC Obtained Peaks of Pesticides

Peak	Retain. Time	Height	Area	Concentration
1	1.092	23	82.9	1.1967
2	1.768	261	6405.7	13.5796
3	2.268	54	378.2	2.8096
4	2.912	210	2042.2	10.9261
5	3.203	1009	11936.6	52.4974
6	4.030	21	294.6	1.0926
7	5.665	199	2523.1	10.3538
8	6.058	145	1701.5	7.5442

Note: Peak 2, 4, 5, 7 and 8 are the major pesticides

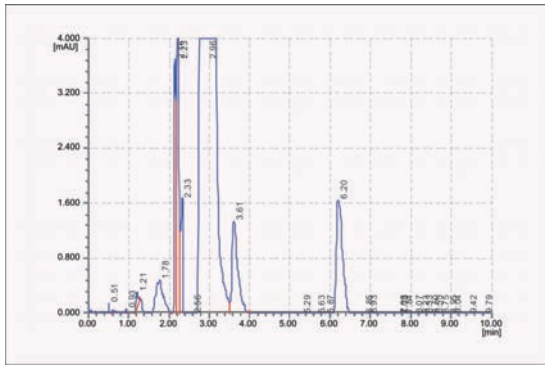

Fig. 2. HPLC profile of Marham Kafoor

Table 6: HPLC Obtained Peaks of Marham Kafoor

Peak	Retain. Time	Height	Area	Concentration
1	0.510	19	26.8	0.118
2	0.927	13	28.1	0.081
3	1.214	23	73.8	0.143
4	1.780	53	723.1	0.330
5	2.115	374	1056.5	2.327
6	2.225	517	1944.9	3.217
7	2.325	292	1319.1	1.817
8	2.559	300	2542.1	1.867
9	2.958	13845	202295.7	86.154
10	3.612	226	3043.2	1.406
11	5.285	13	19.1	0.081
12	5.627	12	70.3	0.075
13	5.868	15	105.7	0.093
14	6.202	183	2219.5	1.139
15	6.856	13	58.1	0.081
16	6.930	14	33.2	0.087
17	7.675	10	19.7	0.062
18	7.725	10	18.9	0.062
19	7.842	10	20.3	0.062
20	8.067	11	17.8	0.068
21	8.233	14	51.6	0.087
22	8.325	11	25.9	0.068
23	8.495	24	47.0	0.149
24	8.553	11	20.4	0.068
25	8.745	13	77.8	0.081
26	8.954	11	27.1	0.068
27	9.037	10	28.8	0.062
28	9.415	11	15.2	0.068
29	9.790	12	94.4	0.075

Vanillin sulfuric acid **Iodine vapour**

Fig. 3. Thin Layer Chromatography of Marham Kafoor

Acknowledgement

Authors are thankful to the Unani Pharmacopoeia Committee and Department of AYUSH, New Delhi for the financial support.

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