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RESEARCH ARTICLE

Standardization and Safety Evaluation of A Polyherbal Formulation"Kabideen (Syrup)"

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Abstract

Standardization is of growing concern for establishment of a consistent biological activity, chemical profile, safety and quality assurance of traditional drugs. In the present study a polyherbal Unani formulation "Kabideen", manufactured by Dawakhana Tibbiya College, Aligarh Muslim University Aligarh, was taken to establish its physicochemical standardization and to evaluate its safety profile. The various parameters studied in this communication include ash values (total, acid insoluble and water soluble), extractive values (aqueous and alcoholic extract), pH values of 1% and 10% solution, viscosity, specific gravity, refractive index and sugar percentage of the formulation. The qualitative and quantitative estimation of various constituents' i.e. alkaloids, amino acids, flavonoids, glycosides, phenols, proteins, resin and sterols /terpenes and thin layer chromatographic studies were performed.

In addition to these parameters, the safety profile of Kabideen was done to evaluate the presence of heavy metals (lead, mercury, arsenic and cadmium). The microbial loads (total bacterial, total yeast and mould count), pesticidal residues and aflatoxins were also determined in Kabideen syrup.

INTRODUCTION

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. The quality assessment of herbal formulations is of paramount importance to justify their acceptability in the present scenario[1]. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. In India, the Department of AYUSH, Government of India, launched a central scheme to develop standard operating procedures (SOPs) for the manufacturing process and to develop pharmacopoeial standards for traditional preparations. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analysed using sophisticated modern techniques of standardization.

World Health Organization (WHO) encourages, and promotes traditional/herbal remedies in natural health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards[2].

The present study was designed to fix the various physico-chemical, and safety standards of a unani polyherbal formulation "Kabideen syrup" manufactured by a reputed Unani pharmaceutical Dawakhana Tibbiya College,

AMU, Aligarh to assess the genuine quality of the formulation as it is used by traditional practioners for last many decades effectively in liver disorders, which can be used as quality control tool and included in Unani Pharmacopoeia of India.

Materials and Method

Collection and authentication of plant sample:

The ingredients of Kabideen were procured from local market of Aligarh then identified and authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi and the pharmacognosy section of the Department of Ilmul Advia, Aligarh Muslim University. The specimens were kept in the Museum of the Department for future reference.

Kabideen syrup is a polyherbal nonpharmacopoeial formulation mainly prescribed for the management of liver disorders. It comprises of 21 ingredients of herbal raw materials, the details of the herbs are listed below in Table no. I.

Table.I Ingredients of Kabideen

S.NO.	Unani Name	Botanical Name	Part used	Quantity
1	Biranjasif	*Achillea millefolium* Linn.	Top of Flowers	10gm
2	Barg-e-shahattara	*Fumaria officinalis* Linn.	Leaves	6gm
3	Barg-e-Kasaundi	*cassia occidentalis* Linn	Leaves	6gm
4	Tukhm-e Kasni	*Cichorium intybus* Linn.	Seeds and root	10gm
5	Tukhm-e-Bathua	*Chenopodium album* Linn.	Seeds	6gm
6	Tukhm-e-Kasoos	*Cuscuta reflexa* Roxb.	Seeds	6gm
7	Tukhm-e Khayarein	*Cucumis sativus and C. melo*	Seeds	6gm
8	Mako	*Solanum nigrum* Linn	Fruits	10gm
9	Rewand chini	*Rheum emodi* Linn	Rhizomes	7gm
10	Sumbullutteeb	*Nordostachys jatamansi* (D.Don) DC.	Rhizomes	6gm
11	Ood hindi	*Aquillaria agallocha* Roxb.	Roots	6gm
12	Narmushk	*Ochrocarpos longifolius* Benth.&Hook.f.	Buds	6gm
13	Satar Farsi	*Zataria multiflora* Boiss.	Leaves	6gm
14	Ushba	*Smilax regelii* Killip & C.V. Morton	Roots	6gm
15	Khulanjan	*Alpinia galanga* (L.) Wild.	Roots	6gm
16	Chiraita shireen	*Swertia chirata* (Wall.) C.B. Clarke	Leaves	3gm
17	Gul-Surkh	*Rosa damascena* Mill.	Flowers	3gm
18	Gul Nilofar	*Nymphaea alba* Linn.	Flowers	6gm
19	Gul-e-Tisoo	*Butea frondosa* Roxb.	Flowers	10gm
20	Gul-e-Ghafis	*Agrimonia eupatoria* Linn.	Flowers	10gm
21	Bekh-e-Kasni	*Cichorium intybus* Linn.	Roots	6gm

Dose 25-50ml.

Method of preparation

The decoction of the ingredients was poured into a tin-coated vessel and added 2.5 parts of sugar then the vessel was kept on low fire and waited till the required consistency[3].

1. Physicochemical Studies

The following parameters were adopted for the standardization of Kabideen syrup. The data was based on multiple observations.

1.1 Organoleptic Characteristics of Kabideen
The organoleptic charecters of Kabideen syrup were examined where the color, appearance, texture, taste and smell of the sample were seen and tabulated in Table No 1

1.2 Extractive values: The mean percentage of alcoholic and aqueous extracts of Kabideen were found to be 35.12±0.491and 65.2 ±0.750 respectively[5]. (Table No 2)

1.3 Ash Values: The mean percentage of the total ash, acid insoluble ash and water soluble ash were found to be 1.296±0.012, 0.221±0.07and 10.426±0.12 respectively5. (Table No 2)

1.4 pH Values: The mean of the pH value of 1% and 10% solution were found to be 4.76±0.10 and 5.77±0.02 respectively[6]. (Table No 2)

1.5 Refractive index: The mean value of refractive index was found to be 1.46638±0.00[8]. (Table No 2)

1.6 Sugar Percentage: The mean value of sugar% was found to be 65.5±0.24[12]. (Table No 2)

1.7 Specific gravity: The mean value of specific gravity was found to be 1.44974±0.002[4, 5]. (Table No 2)

1.8 Viscosity: the mean value of viscosity was found to be 13.82±0.06[6, 11]. (Table No 2)

1.9 Thin Layer Chromatography: TLC studies of the extracts of test drug (Kabideen) were carried out using different organic solvent systems. The solvent system for Hydroalcoholic extract were Choloform: methanol= 18:1, petroleum ether: diethyl ether = 5:2, n butanol:acetic acid: water= 4:1:5, The Rf values of spots were calculated[4,5]. The readings were tabulated in table No 3-5 and Fig I-III.

1.10 Qualitative analysis of various constituents present in Kabideen was analyzed using different parameters and the results were tabulated in[5, 10](Table No 6)

2. Safety studies

2.1 The heavy metals (lead, arsenic, cadmium, mercury) were determined in the sample either not detected or found within permissible limit[9]. (Table No 7)

2.2 In microbial load Total Bacterial Count, Total Yeast & Mould Count were evaluated in the Kabideen and found to be within the permissible limits. Specific Pathogen likes E.Coli, Salmonella, S. aureus, P. aeruginosa were found to be absent[9] (Table No 8).

2.3 Mycotoxin (aflatoxinB1, aflatoxinB2, aflatoxinG1, aflatoxinG2) was also tested and not detected in the test drug[13]. (Table No 9)

2.4 In pesticidal residue determination the organochlorine pesticides like DDT, endosulafan and Organophosphorus Pesticides like Chlorpyriphos, Malathion, and Parathion were not detected[7]. (Table No 10)

Result and discussion

Table-1: Organoleptic Description of kabideen

Colour	Brown
Appearance	Syrup
Texture	Liquid
Taste	Mildly bitter
Smell	Agreeable

Table-2: **Physico-Chemical Analysis of Kabideen**

S. NO.	PHYSICO-CHEMICAL PARAMETERS	MEAN±S.E.
1.	**Sugar (%)**	65.5
2.	**Extractive values (%)**	
	Alcohol soluble extract Water soluble extract	35.12±0.491 65.5±0.750
3.	**Ash Value (%)**	
	Total Ash Acid insoluble Ash Water soluble Ash	1.296±0.01 0.22±0.07 10.426±0.12
4.	**Refractive index**	1.46638±0.00
5.	**pH Values**	
	pH of 1% solution pH of 10% solution	4.76±0.01 5.77±0.02
6.	**Specific Gravity**	1.44974±0.002
7.	**Viscosity**	13.82±0.06

Table-3: TLC Profile of Hydroalcoholic extract of Kabideen using solvent system as n-butanol:acetic acid and water

Treatment	Mobile phase n butanol : acetic acid : water (4:1:5)	
	No of spots	**Rf value and colour of spots**
Day light	0	0
UV short	3	0.96, 0.53, 0.13
UV long	5	0.96 (red),0.68(sky blue), 0.53(red), 0.5(sky blue), 0.13(blue)
Iodine vapour	1	0.96(brown)

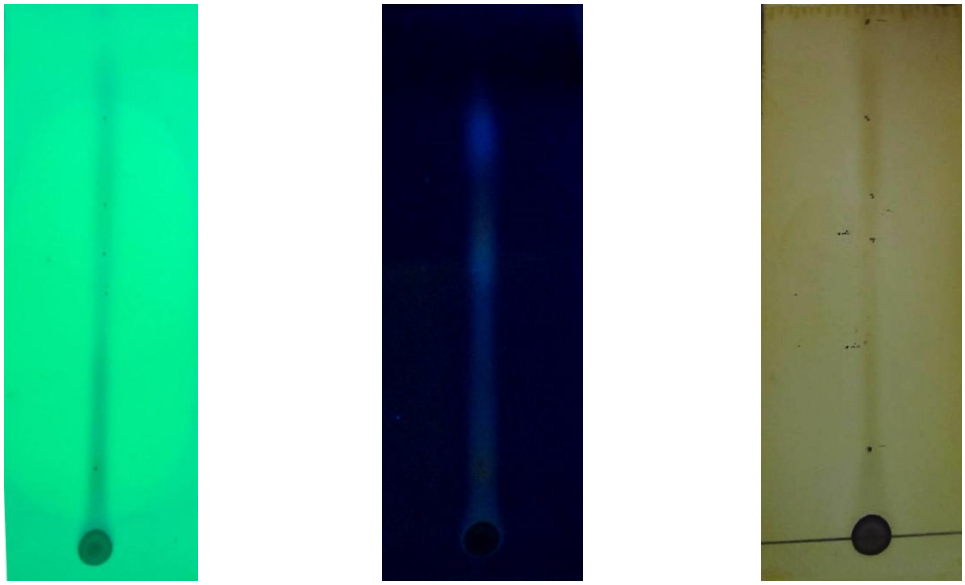
Table-4: TLC Profile of Hydroalcoholic extract of Kabideen using solvent system as chloroform: Methanol

Treatment	**Mobile phase Chloroform:Methanol (18:1)**	
	No of spots	**Rf value and colour of spots**
Day light	1	0.96(brown)
UV short	5	0.96,0.74,0.49,0.34,0.30
UV long	7	0.96(blue),0.49(blue), 0.34(red), 0.30(red), 0.16(pink), 0.12(blue), 0.05(blue)
Iodine vapour	1	0.96(brown)

Table-5: TLC Profile of Hydroalcoholic extract of Kabideen using solvent system as petroleum ether: diethyl ether

Treatment	**Mobile phase petroleum ether: diethyl ether (5:2)**	
	No of spots	**Rf value and colour of spots**
Day light	1	0.41(light yellow)
UV short	3	0.41,0.28,0.16
UV long	1	0.28(sky blue)
Iodine vapour	1	0.04(brown)

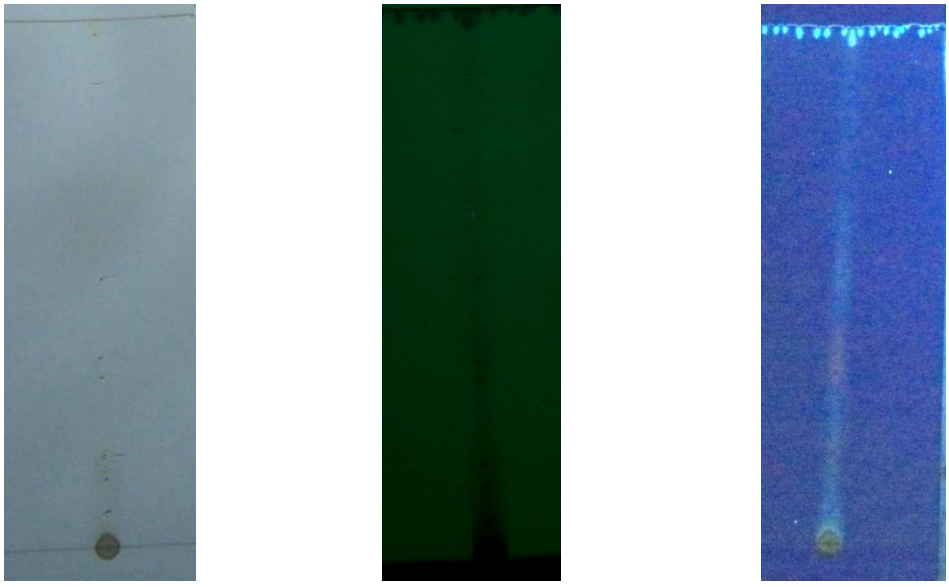
Fig I. TLC Profile of Hydroalcohalic extract of Kabideen



UV Short **UV Long** **Iodine Vapour**

(n Butanol : Acetic acid : Water 4: 1: 5)

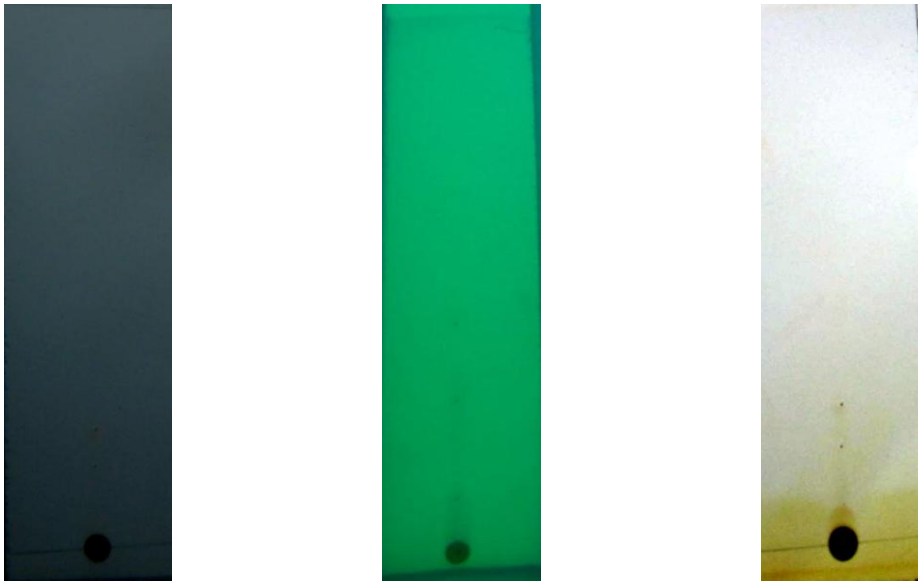
Fig II. TLC Profile of Hydroalcoholic extract of Kabideen



Day Light **UV Short** **UV Long**

(Chloroform: Methanol 18:1)

Fig III. TLC Profile of hydroalcoholic extract of Kabideen



Day Light **UV Short** **Iodine Vapour**

(Petroleum ether: Diethyl ether 5: 2)

Table-6: Qualitative Test for Kabideen

S.No.	Constituents	Result
1.	Alkaloids	+ve
2.	Amino acid	-ve
3.	Protein	-ve
4.	Glycosides	-ve
5.	Flavonoids	+ve
6.	Phenols	+ve
7.	Resin	-ve
8.	Sterol/ Terpene	+ve
9.	Tannin	+ve

Table-7: Heavy Metals in Kabideen

S. No	Heavy Metals	Result (in mg/ kg)	LOQ(inmg/kg)	Permissible limit(in mg/kg)
1	Lead (Pb)	Not detected	0.03	NMT 10
2	Mercury (Hg)	Not detected	0.03	NMT 1
3	Arsenic (As)	Not detected	0.03	NMT 3
4	Cadmium (Cd)	Not detected	0.03	NMT0.3

Table-8: Microbial load in Kabideen

S. No.	Microbes	Result	Permissible Limit
1	Total Bacterial Count	10000 cfu/g	NMT 10^5cfu/g
2	Total Fungal Count	<10 cfu/g	NMT 10^3cfu/g
3	Enterobacteriaceae	absent	absent
4	Salmonella	absent	absent
5	Staphylococcus aureus	absent	absent

Table-9: Aflatoxins in Kabideen

S. No.	Aflatoxins	Result	LOQ(inmg/kg)	Permissible Limit (in mg/kg)
1	Aflatoxin B1	Not detected	0.001	NMT 0.50
2	Aflatoxin G1	Not detected	0.001	NMT 0.50
3	Aflatoxin G2	Not detected	0.001	NMT 0.10
4	Aflatoxin B2	Not detected	0.001	NMT 0.10

Table-10: Pesticidal residues in Kabideen

S.No	Pesticidal Residues	Result (inmg/kg)	LOQ (inmg/kg)	Permissible Limits(inmg/kg)
1	Chlorpyriphos	Not detected	0.01	0.2
2	Chlorpyriphos-methyl	Not detected	0.01	0.1
3	DDT (Sum of p.p-DDT, p.p-DDE and p.p-TDE)	Not detected	0.01	1.0
4	Endosulfan (Sum of Isomer and Endosulfan Sulphate)	Not detected	0.01	3.0
5	Malathion	Not detected	0.01	1.0
6	Parathion	Not detected	0.01	0.5
7	Parathion Methyl	Not detected	0.01	0.2

The efficacy of the herbal drug mainly depends upon the physical and chemical properties; therefore the determination of physichochemical characters of test drug Kabideen was necessary for the purpose of authentication and quality assurance also.

The polyherbal formulation Kabideen (Syrup) was slightly brown colour, mildly bitter in taste with characteristic agreeable odour. Physio-chemical parameters of test sample are tabulated in (Table-2) Total ash value of plant material indicates the amount of minerals and earthy materials present in the drug. Analytical results showed total ash value of Kabideen as 1.296±0.01 %. The amount of acid-insoluble ash and water soluble ash present in the formulation was 0.221±0.07 % and 10.426±0.12% respectively. The water-soluble extractive value indicates the presence of sugar, acids and inorganic compounds. Less or more extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying, or storage of formulation. The water-soluble extractive value was found to be 65.2±0.750 %. The alcohol-soluble extractive value was found to be 35.12±0.491 %. The observed pH values of 1% and 10% solutions of the formulation were found to be 4.76±0.01 and 5.77±0.02 respectively which indicate suitability for human use. The viscosity of solutions and liquid mixtures indicates the composition and rapid means of analysis. The value of viscosity was found to be 13.82±0.06 stokes. Specific gravity indicates of the identity and purity of the substance. The value of specific gravity was found to be 1.499±0.002. Refractive index shows the identity and purity of drug and food products. It may be used to determine quantitatively the strength and purity of solution. The refractive index was found to be 1.466±0.00. Sugar percentage of syrup indicates the safety from fungal and other infections. Sugar percentage of Kabideen was found to be 65.5±0.24. Thin layer chromatography was done for detecting the adulteration and judging the quality of the test drugs. The different kinds of chemical components were separated by using TLC and calculating the R_f values after detecting the spots in order to standardize the drug for its identity, purity and strength. R_f values and number of spots seen during study were recorded and tabulated in (Table 3-5). Qualitative phytochemical study of Kabideen revealed the presence of carbohydrate, tannin, alkaloid, phenol, and sterol and mentioned in Table 6. The presence of these active constituents plays an important role in describing the therapeutic properties of Kabideen.

As per WHO guidelines (1998) the determination of heavy metals, pesticidal residue, Aflatoxins, microbial load is exclusively very important to be carried out in all herbal formulations because they may cause serious adverse effects in human being if they are contaminated with toxic substances hence safety study was also performed in Kabideen

Aflatoxins, pesticides residue, heavy metals and microbial loads were not detected or found within the permissible limits (Table 7-10). All the above findings show that the sample is genuine, free from adulteration and satisfied the need of quality control and could be safely used in human beings.

Conclusion

It was concluded that the data analysed during safety study of Kabideen such as the sugar percentage, alcohol soluble, and water soluble extractive value, refractive index, specific gravity, viscosity, pH, total ash, water-soluble ash, acid-insoluble ash, qualitative analysis of chemical constituents, TLC and organoleptic characteristics could be recognized as standard parameters. Physico-chemical studies must be included in Unani Pharmacopoeia/Formularies. In addition to the above parameters, the safety profile of kabideen was also done to evaluate the presence of heavy metals (lead, mercury, arsenic and cadmium) to count the microbial loads (total bacterial, total yeast and mould count) and to estimate the pesticidal residue and aflatoxin in kabideen syrup they could be considered as an important parameter for safety evaluation and can be efficiently used for standardization of this polyherbal Unani formulation. All results tabulated in table 7-10 they were either not detected or found within permissible limits, therefore the study should be utilized as reference standard for the quality control and quality assurance of Kabideen and setting limits.

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