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STANDARDIZATION OF KAMELA POWDER (*MALLOTUS PHILIPPINENSIS*): AN IMPORTANT HERBAL UNANI DRUG

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ABSTRACT

Kamela (*Mallotus philippinensis*) belongs to the family Euphorbiaceae. It is also known as Monkey Face tree. It is a prime drug of Unani Medicine commonly used in the treatment as an anthelmintic, purgative, Tapeworm, Ringworm, Various skin problems and colicky pain, urinary and menstrual disorders etc. Due to natural variations a number of natural products have significantly different biological activity and varied clinical efficacy. Therefore, it becomes essential to standardize the herbal drugs to ensure their identity, quality and purity so as to ascertain their therapeutic efficacy. In the present study an attempt has been made to determine the physicochemical characters helpful in identification, standardization and quality control of Kamela. It includes the parameters used in National Unani Pharmacopeia i.e. Total Ash values, Acid insoluble ash, Water soluble ash, successive extractive values and moisture content. Qualitative analysis and Chromatographic study (TLC) were also performed.

KEYWORDS: Standardization, Kamela (*Mallotus philippinensis*), Quality analysis, TLC.

INTRODUCTION

Medicinal plants have always had an important place in the therapeutic armory of mankind.[1] According to WHO, 60% of world population relay on the medicinal plants for their primary health care needs.[2] Over the 50% of all the modern clinical drugs are of natural product origin and natural products play an important role in the drug development programs of the pharmaceutical industry[3] so, while developing an herbal drugs formulation it must to have all the related knowledge of all its organoleptic characters, phytoconstituents, pharmacological action to its standardization in respect to various parameters via various technique. *Mallotus philippinensis* also known as Kamela consists of dried seed trichomes and glands separated from the fruit of *Mallotus philippinensis.*[4,5] This is small evergreen shrubs and tree with a thin grey bark, variable leaves, flower in spikes, and globose capsules.[6,7] the tree produces three-celled capsular fruit about the size of a large pea, and more or less completely covered with red powder. Fruits are gathered during the month of March.[8,9] various part of the plant are used in the treatment of skin problem, antifungal tapeworm diarrhoea urogenital infection etc.[10] extract of the fruit of kamela from the glands and hairs yield the crystalline compound rottlerin. Its fruit contains rottlerin, fixed oil, oleic, lauric, myristic, palmitic acid, iso rottlerin, homorottlerin tenins, citric acid and oxalic acids.[1,11] Despite of the moderns' techniques, identification and evaluation of the plant's drugs by pharmacognostical studies is still more reliable and inexpensive. According to WHO the pharmacognostical evaluation first step towards the identity purity hence objective of this study is to provide the reference information for identification and preparation of plant monograph that can be used to study the quality and purity of the drugs.[12]

MATERIAL AND METHOD

Collection of Drug Materials

The drugs sample of kamela *(Mallotus philippinensis)*, were purchased from Dawakhana Tibbiya College A.M.U, Aligarh. The ingredients were identified and authenticated by the pharmacognosy section of the Department of the Ilmul Advia, Faculty of Unani medicine A.M.U Aligarh, and found within the range of the standards. The specimen voucher no. SC-0238/18 for kamela *(Mallotus philippinensis)*.

Determination of Organoleptic Characteristics

Organoleptic evaluation refers to evaluation of the drug by its appearance, colour, odour, taste and texture.

Physicochemical Study

The Physicochemical study included the determination of extractive values of the test drug in different solvents, ash values and moisture content.

Ash values

Total Ash

About 2 to 3 gm accurately weighed powdered drug was incinerated in silica dish at a temperature not exceeding 45^0C, until free from carbon. It was then cooled and

weighed. The percentage of ash was calculated with reference to air dried drugs.[13]

Water Soluble Ash
The ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 45^0C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represented the water soluble ash.

The percentage of water soluble ash was calculated with reference to air dried drugs.[13]

Acid Insoluble Ash
The ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.[13]

Moisture Content
The drug was kept in a flask along with sufficient quantity of toluene. The level of toluene was kept above the level of the drug to allow the later to get submerged. Then it was distilled for sufficient time. The distillate was collected in a measuring receiver along with the toluene, and a separated upper layer was measured in the receiver.[14]

Successive extractive values
It measures the amount of a certain constituent or a group of related constituents in a particular solvent, the drug contains. The drug was extracted in different solvent in order of ascending polarity by using Soxhlet apparatus.[14]

Qualitative Analysis
The qualitative analysis of different chemical constituents, present in test drug was carried out according to the scheme proposed by Bhattacharjee and Das (1969).[15]

Test for Alkaloids
A drop of Dragendroff's reagent was added in the extract. The brown precipitate showed the presence of alkaloids.

Test for Carbohydrate / Sugars
Fehling's Test
In the aqueous extract, a mixture of equal parts of Fehling's solution A and B previously mixed, was added and heated. A brick red precipitate of cuprous oxide indicates the presence of reducing sugars.

Molisch test
In an aqueous extract, α-napthol was added. Afterwards, concentrated sulphuric acid was gently poured. A brown colour ring at the junction of the two solutions indicates the presence of the sugar.

Test for Flavonoids
A piece of Magnesium ribbon was added to the alcoholic extract of the drug followed by drop wise addition of concentrated HCl. Colour ranging from orange pink to red is a confirmatory test for flavonoids.

Test for Glycosides
The test solution was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with magnesium oxide. The remaining alcoholic extract that contained the glycosides was subsequently detected by the following method.

The hydrolysis of the solution was done with concentrated sulphuric acid and after the hydrolysis sugar was determined with the help of Fehling's solutions.

Test for Tannin
Ferric chloride solution was added in the aqueous extract of the drug. A bluish-black colour, which disappeared in addition to dilute sulphuric acid followed by a yellowish brown precipitate, shows the presence of tannin.

Test for Proteins
Xanthoproteinic reaction
In the test solution, concentrated nitric acid was added. A yellow precipitate appeared. Strong solution of ammonia was added to it. Appearance of yellow colour shows the presence of proteins.

Biurette´s reaction
In the hot test solution, 1ml concentrated sodium hydroxide was added, followed by one drop of copper sulphate solution. A violet or red colour indicates the presence of proteins.

Test for Amino Acids
The alcoholic extract was mixed with ninhydrin solution (0.1% in acetone). After heating gently on a water bath for few minutes it gives a blue to red-violet colour that indicates the presence of amino acids.

Thin Layer Chromatography (TLC)
Thin Layer Chromatography of different extract was carried out on T.L.C. pre-coated aluminium plates (silica gel 60 of F_{254} layer thickness 0.25 mm) for all extracts in various phases later sprayed by different spraying reagents. The Rf values of the spots were calculated by the following formula.[14,16]

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

RESULTS
The Organoleptic evaluation carried out has been given below in table 1.

Table 1: Organoleptic characters.

S. NO.	Organoleptic characters	Observations
1.	Appearance	Red Powder
2.	Colour	Red
3.	Odour	Odourless
4.	Texture	Firm and smooth
5.	Taste	Tasteless

Physicochemical parameters

The Physicochemical parameters carried out has been given below in table 2

Table 2: Physicochemical parameters.

S.NO.	Parameters	Results
1.	Ash value	Total Ash: 3.432±0.046 Water soluble: 4.043±0.0147 Acid Insoluble Ash: 4.419±0.073
2.	Moisture content	4.132±0.016
4.	Extractive values	Alcohol soluble: 61.010±0.849 water soluble: 0.95±0.787

Qualitative analysis

The results are shown in table 3.

Table-3: Qualitative analysis of Kamela *(Mallotus philippinensis)*.

S. No.	Chemical Constituent	Test Reagent	Inference
1.	**Alkaloids**	Dragendorff's reagent	+ve
		Mayers' s reagent	+ve
2.	**Amino acid**	Ninhydrin solution	+ve
3.	**Carbohydrates**	Fehling Test	+ve
		Benedict test	+ve
4.	**Flavonoids**	Mg Ribbon and dil. Hcl	+ve
5.	**Glycosides**	NaOH Test	+ve
6.	**Phenols/Tannins**	Ferric Chloride Test	+ve
		Lead Acetate Test	+ve
7.	**Proteins**	Biuret Test	+ve
		Xanthoproteic Test	+ve
8.	**Saponins**	Frothing with $NaHCO_3$	+ve

Thin Layer Chromatography (TLC)

TLC carried out on already prepared plates for different successive extracts viz. pet. Ether and alcohol in different mobile phases and spray/treatment where number of spots and Rf values were noted. (Table-4-a, b, c and d Figure-1- a, b, c and d)

Table-4 (a): Thin Layer Chromatography of Petroleum ether extract of Kamela (*Mallotus philippinensis*).

Treatment	Solvent System as petroleum ether : Benzene (9:1)	
	No of spots	Rf value and colour of spots
Day light	3	0.1 (brown), 0.25(yellow brown), 0.33 (yellow),
UV short	3	0.09 (blackish brown), 0.4 (greenish yellow), 0.5 (light blue)
UV long	3	0.1 (black), 0.25(blackish blue), 0.33 (blue),

Table-4 (b): Thin Layer Chromatography of Petroleum ether extract of Kamela (*Mallotus philippinensis*).

Treatment	Solvent System as Petroleum ether : ether(4:1)	
	No of spots	Rf value and colour of spots
Day light	3	0.1 (brown yellow), 0.2 (yellow), 0.33 (yellow)
UV short	5	0.11 (dark brown), 0.17 (blue), 0.2 (light blue), 0.33(greenish yellow), 0.35 (blue)
UV long	3	0.44 (black), 0.11 (blackish brown), 0.33 (blackish blue),

Table-4 (c): Thin Layer Chromatography of Alcoholic extract of Kamela (*Mallotus philippinensis*).

Treatment	Solvent System as Benzene :Acetic Acid (15:1)	
	No of spots	Rf value and colour of spots
Day light	7	0.26 (red), 0.46 (blue), 0.6 (reddish), 0.72 (light brown) 0.81 (brown), 0.92 (brick red) 0.97), (radish brown)
UV short	7	0.26 (light blue), 0.47 (light brown), 0.64 (blue), 0.73 (yellow), 0.80 (brownish), 0.93 (brick red), 0.98 (radish brown)
UV long	7	0.26 (red), 0.46 (blue), 0.6 (reddish), 0.72 (light brown) 0.81 (brown), 0.92 (brick red) 0.97), (radish brown)

Table-4 (d): Thin Layer Chromatography of Alcoholic extract of Kamela (*Mallotus philippinensis*).

Treatment	Solvent System as Chloroform : Methanol acid (1:1)	
	No of spots	Rf value and colour of spots
Day light	7	0.33 (light blue), 0.59 (light brown), 0.67 (blue), 0.82 (yellow), 0.89 (brownish), 0.97
UV short	7	0.33 (light blue), 0.59 (light brown), 0.67 (blue), 0.82 (yellow), 0.89 (brownish), 0.97
UV long	7	0.33 (light blue), 0.59 (light brown), 0.67 (blue), 0.82 (yellow), 0.89 (brownish), 0.97

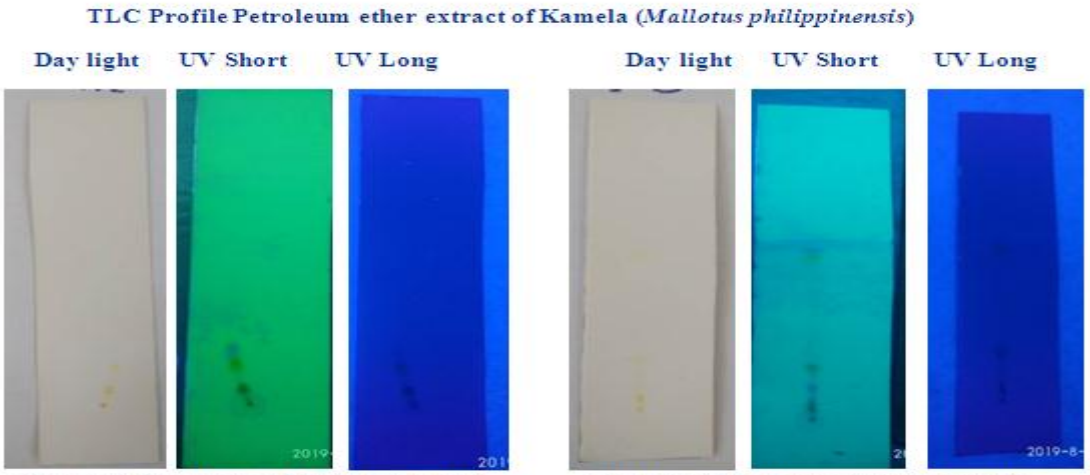

Fig-1: (a) Petroleum ether: Benzene (9:1) Fig-1 (b) Petroleum ether: ether (4:1)

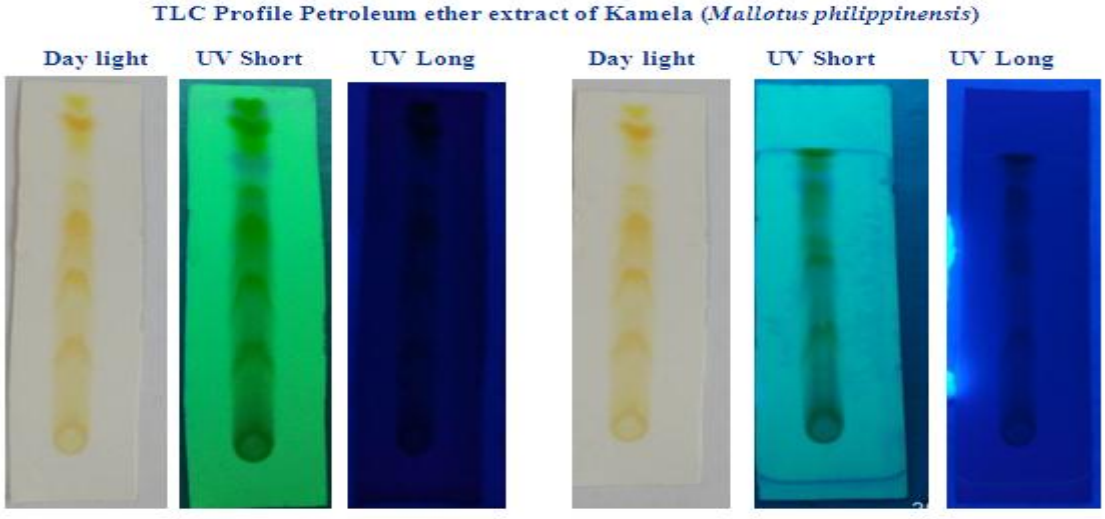

Fig-1 (c) Benzene: Acitic Acid (15:1) Fig-1 (d) Chloroform: Methanol acid (1:1)

DISCUSSION

Standardization is an essential measurement for ensuring the quality control of the herbal drugs. India can emerge as the major country and play the lead role in the production of standardized, therapeutically effective Unani drugs and its formulations. India needs to explore

the medicinally important plants. Standardization of herbal formulation is essential in order to assess the quality of drugs, based on the concentration of their active principles. It is an essential tool to ensure identity, purity and quality of herbal drugs. Pharmacognostical studies are the first step of standardization which helps in identification, characterization and distinguishing the drug from confounding varieties. Since the therapeutic efficacy of a drug mainly depends upon its physicochemical characteristics therefore, the determination of physicochemical characters for the authenticity of a drug is imperative before studying it for pharmacological activity. Physicochemical study helps in characterization of constituents or groups of constituents which interact at molecular level in human beings.

Standardization of Kamela *(Mallotus philippinensis)* which is an effective anthelmintic drug will ensure its proper identification, purity and quality and thereby its therapeutic efficacy. The findings of the present study will also help in distinguishing it from similar varieties which possess few common characters. The present study determines a comprehensive range of physicochemical characters of the drug according to the parameters used in National Formulary of Unani Medicine. Therefore, these findings may be used as the standards for ensuring the purity and quality and thereby the predictable efficacy and safety of Kamela *(Mallotus philippinensis)*. The generated information of the present study will provide data which is helpful in the correct identification & authentication of this medicinal plant and may help in preventing its adulteration. Morphological evaluation plays the role of determining the stability of crude drugs in the market. It is the simplest method for identification to start the correct identity.[17] The phytochemical content of the dried fruit powder is moisture content, ash values, and extractive values.[18] The limited moisture content signifies that the drug was properly dried and rate of drying was good enough. The ash value reports the inorganic salts in the crude drugs. It shows that the adulterant is present in the crude drugs.[12,17] The present of the extractive value in the different solvent shows the results of the physicochemical contents of the dried fruits powder which lies within the limits; this significant that the quality and purity of raw material was within the limits.

CONCLUSION

This study would provide reference information for identification and preparation of plant monograph that can be used to study the quality and purity of this drug.

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