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STANDARDIZATION OF KHULANJAN (*ALPINIA GALANGA*): AN IMPORTANT HERBAL UNANI DRUG

Mokarram Ali[1]*, Naeem A. Khan[2] and Sawood Ahmad[3]

Department of Ilmul Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh (UP), 202002.

***Corresponding Author: Dr. Mokarram Ali**

Department of Ilmul Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh (UP), 202002.

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ABSTRACT

Khulanjan is the (rhizome of *Alpinia galanga* (L) wildly used in Traditional Medicine in various diseases and as one of the constituents of many preparations. It belongs to the family Zingiberaceae. It is one of the most potent drugs that have been used since long time in Unani System of Medicine for the management of diabetes mellitus. This drug has been described in Unani Classical literatures having astringent property and used in the management of renal colic and other ailments. Khulanjan also produces hypoglycaemic effect when used in different animal species. It also has aphrodisiac, nervine tonic and stomachic properties. Due to different place of cultivation and Environmental condition i.e natural variations a number of natural products have significantly different biological activity and varied clinical efficacy. Therefore, it becomes imperative 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 Khulanjan. It includes the parameters used in Unani Pharmacopeia of India i.e. Ash values (Total ash, acid insoluble ash, water soluble ash), successive extractive values , loss on drying, pH at 1% & 10%, bulk density (poured & tapped density) and moisture content. Qualitative analysis and Chromatographic study (TLC) were also performed.

KEYWORDS: Standardization, Khulanjan, safety of *Alpinia galanga*, TLC.

INTRODUCTION

Khulanjan is the (rhizome of *Alpinia galanga* (L) wildly used in Traditional Medicine in various diseases and as one of the constituents of many preparations. It belongs to the family Zingiberaceae. It is one of the most potent drugs that have been used since long time in Unani System of Medicine in the management of diabetes mellitus. This drug has been described in Unani Classical literatures having astringent property and used in the management of renal colic and other ailments.[1,2,3,4] reported that Khulanjan produces hypoglycaemic effect when used in different animal species.

The word Alpinia is given by Plumier in honour of Frospero Alpino an eminent Italian botanist. The Genus Alpinia comprises of 40 species which belongs to the family Zingiberaceae. Among them 17 species are found in India and some are used for medicinal purposes. The *Alpinia galanga* (Greater galangal) is important one.

The greater galangal and lesser galangal both are known in China by the name of **Kaon Leang** and **Liang Keang.** From the first of these names the Arab have derived their name **Khulanjan** or **Khowlanjan**, which is applied to the greater galangal and the lesser galangal and is the source of European names for these drugs. The earliest notice of the drug occur in Persian literature (cf. Burhan) where it is stated **"Khurudaru"** Choros remedy was introduced in the time of **Noshirwan** (5^{th} Century). It probably reached Persia by the Central Asia trade route. The Hindus first become acquainted with Chinese galangal through Arab and they call greater galangal with the name **Malabari Vacha** of the **Bhavaparkash** and from the name it appears that Hindus regard the plant as a native of Malabar or of Western India. The Persians, Greeks and Arabs had not distinguished between greater and lesser galangal.

It also used in India by Hindu and Muslim Physicians from time immemorial.[5,6] Present study has been designed to study on Khulanjan, (*Alpinia galanga*) for certain physicochemical parameters in order to set the standards of its quality and purity.

MATERIAL AND METHODS

Khulanjan (*Alpinia galanga*) was procured from the Dawakhana Tibbiya College, A.M.U, Aligarh. The Pharmacognosy Section Department of Ilmul Advia, A. K. Tibbiya College, Aligarh Muslim University, and Aligarh identified the drug sample. The sample of the test drug was submitted to Mawalid-e-Salasa Museum of

the Department for future reference with the voucher No of Sc-0245/18.

The rhizome of Khulanjan (*Alpinia galanga*) was ground to get coarse powder. The powder was then subjected to physicochemical and phytochemical studies to determine various constants.

Determination of Organoleptic Characteristics
Organoleptic evaluation refers to evaluation of the drug by its appearance, colour, odour, taste and texture (Table-1).

Physicochemical Study
The Physicochemical study included the determination of extractive values of the test drug in different solvents, moisture content, ash values, loss of weight on drying, bulk density and pH values (Table-2).

ASH VALUES
Total Ash
About 2 to 3 gm accurately weighed powdered drug was incinerated in silica dish at a temperature not exceeding 450C, until free from carbon. It was then cooled and weighed. The percentage of ash was calculated with reference to air dried drug.[7]

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 450C. 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 drug.[7]

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.[7]

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 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.[8]

Loss of Weight on Drying
The known weight of the test drug was taken, spread uniformly and thin layered in a shallow Petri dish. It was heated at a regulated temperature of 105^0C, cooled in a desiccator and weighed. The process was repeated many times till two consecutive weights were found constant. The percentage of loss in weight was calculated with respect to initial weight.[9]

PH Value
Determination of pH was carried out by a synchronic digital pH meter (model no. 335) equipped with a combined electrode. The instrument was standardized by using buffer solution of 4.0, 7.0, and 9.20 to ascertain the accuracy of the instrument prior to the experiment. The pH value of 1% and 10% aqueous solution of powdered drug was measured.[7]

Bulk Density
It was measured by Digital Bulk Densitometer. A clean, dry and previously washed bottle of 250 ml capacity was filled with 100 gm of powdered test drug. It was allowed to tap till the time when no further decrease in level of drug was observed. It was calculated by the formulae-
Poured Bulk Density = Mass of powdered drug / Volume (poured) of test drug.
Tapped Bulk Density = Mass of powdered drug / Volume (tapped) of test drug.[9]

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). The powder of the test drug was extracted with petroleum ether (BP.60-80^{0}C). The petroleum ether extract (I) was tested for free phenols, alkaloids and sterols/terpenes. A part of this extract was saponified and this portion (II) was tested for fatty acids, whereas, unsaponified portion (III) was tested again for phenols, and sterols/terpenes for confirmation. The defatted marc was divided into two portions. One portion was extracted with hot water and the other with ethanol (70%). The aqueous (IV) and alcoholic (V) extracts were tested for alkaloids, flavonoids, saponins, sugars and tannins. Aqueous extract was extracted with ether and ether soluble portion (VI) was tested again for alkaloids, sterols/terpenes, whereas, water-soluble portion (VII) was tested for glycosides. The water-soluble portion was again hydrolyzed with 5% hydrochloric acid and extracted with chloroform. The aglycone portion (VIII) was tested for insoluble hydrochloride of alkaloid. Chloroform soluble portion (IX) was tested for alkaloids and sterols/terpenes, whereas water-soluble fraction (X) was tested for alkaloids. One part of this water-soluble portion was basified with alkali (ammonia) and extracted with immiscible solvent (ether). The solvent soluble part (XI) was again tested for alkaloids.[8] (Table-3).

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 on addition of 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 STEROL/TERPENES

Salkowski reaction

In the test solution of chloroform 2 ml sulphuric acid (concentrated) was mixed from the side of the test tube. The colour of the ring at the junction of the two layers was observed. A red colour ring indicates the presence of the sterols/terpenes.

Test for Amino Acids

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

Thin Layer Chromatography

Thin Layer Chromatography of petroleum ether extract of drug was carried out on aluminium plates precoated with Silica gel-G (Layer thickness 0.20-0.25 mm) for all extracts in various phases later sprayed by different spraying reagents. The Rf value of spots was calculated by the following formulae.[9]

Rf Value - Distance travelled by the spot / Distance travelled by the solvent

OBSERVATIONS AND RESULTS

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

Table 1: Organoleptic characters.

S.NO.	Organoleptic characters	Observations
1.	Appearance	Large rhizome (having uneven surface)
2.	Colour	Brown colour
3.	Odour	Pungent
4.	Texture	Firm and rough
5.	Taste	Spicy

Table 2: Physicochemical parameters.

S.NO.	Parameters	Results
1.	Ash value	Total Ash: 5.56 ±0.2028 Water soluble: 3.2±0.052 Acid Insoluble Ash: 1.05±0.017
2.	Moisture content	1.23±0.0164**Error! Filename not specified.**
3.	Bulk density: Poured Density Tapped Density	0.8±0.0142 0.57±0.0164
4.	Loss on drying at 105^0C	6.37±0.2082
5.	pH values	1 % pH- 6.6 ± 0.1049 10 % pH- 5.4 ± 0.3606
6.	Extractive values	**Error! Filename not specified.** Petroleum ether 2.4±0.0515 Diethyl ether 3.1±0.042 Chloroform 7.7±0.0342 Acetone 5.1±0.213

		Alcohol Distilled water	3.32±0.064 10.4±0.256

Table 3: Qualitative analysis of Khulanjan (*Alpinia galanga*)

S.NO.	Chemical constituents	Tests/reagent	Inference
1.	Alkaloid	Dragendroff´s reagent	-
		Hager´s test	-
		Mayer´s reagent	-
2.	Carbohydrate	Molisch´s Test	+
		Fehling´s test	+
3.	Glycoside	NaOH Test	+
4.	Flavanoids	Mg ribbon and Dil. Hcl	+
5.	Tannin	Ferric chloride test	+
6.	Protein	Xanthoproteinic test	+
		Biurette´s test	+
7.	Steroid	Salkowski reaction	+
8.	Amino acid	Ninhydrin solution	+

The Qualitative test for chemical constituents demonstrated that alkaloids, glycosides, flavonoids, proteins, amino acids, tannins and steroids were present in Khulanjan (*Alpinia galanga*).

Table 4: TLC profile of Khulanjan (*Alpinia galanga*).

Treatment	Mobile Phase	No of spots	Rf values and colour of spots
Petroleum ether extract			
Day light	(a) Petroleum ether: Ether 4:1	2	.42 (BW), .92 (FW)
UV short		2	.42 (BW), .92(FW)
UV Long		2	.42(BW), .92(FW)
Iodine vapours		2	.42(BW), .92(FW)

B= blue, W= white, F=florescence

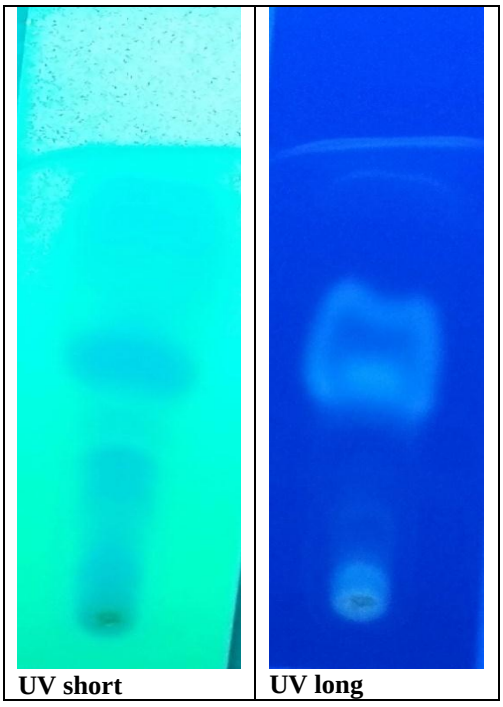

Fig. 1: TLC of Petroleum ether extract of Khulanjan (*Alpinia galanga*).

DISCUSSION

Standardization is an essential measurement to maintain quality control and world wide acceptance of herbal drugs. India can become known as the major country and play the lead role in the production of standardized, therapeutically effective Unani drugs and its formulations. India want 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 standardisation which are very helppfull for 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 Khulanjan (*Alpinia galanga*) which is an effective aphrodisiac 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 Khulanjan (*Alpinia galanga*). 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.

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