Research Article

Exploration of physicochemical and phytochemical potential of *Linum usitatissmum* Linn (Tukhm-e-Katan)

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Abstract

Background: Unani Medicine possess a large number of drugs used in various diseases as mentioned by eminent Unani Physicians based on their own long term experience. But, a doubt always remains regarding the standardization of Unani drugs. **Objective:** Therefore, the present study was aimed to standardize and to assure the quality control check of an important Unani drug Tukhm-e-Katan used for various infectious diseases. **Material and Methods:** The test drugs, Tukhm-e-Katan (*Linum usitatissmum* L.) were procured from local market of Aligarh. The Physicochemical study includes the parameters recommended by National Unani Pharmacopeia Committee, these parameter are the determination of organoleptic characters, extractive values of the test drug in different solvents, alcohol and water soluble contents, moisture content, ash values, loss of weight on drying, bulk density and pH values, and the preliminary phytochemical screening was carried out with different extract of *Linum usitatissimum* for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods. **Results:** Ash values, Total ash, (3.79%) acid insoluble ash, (2.91%) water soluble ash, (0.96%) Successive extractive values in different solvent; petroleum ether (30.56%), diethyl ether (7.93%), chloroform (4.2%), ethyl acetate (2.9%), acetone (1.7%), alcohol (2.6%), aqueous (8.5%), solubility in alcohol (33.06%) and water (12.26%), loss on drying (7.72%), pH at 1% (7.62),& 10% (6.68), bulk density (0.73%) and moisture content (7.0%). **Conclusion:** Preliminary phytochemical analysis of Linseed (*Linum usitatissimum*) showed presence of Alkaloid, flavonoids, steroids and terpenoids which may be active compound, responsible for its wide activities.

***Keywords*:** Standardization, Flax, Katan, *Linum usitatismum*, Linseed, Tukhm-e-Katan

Introduction

The herbal or natural drugs show significant variation in the chemical composition. This can be so drastic as to cause therapy failure or toxicity, so it can be appreciated that different samples of the same natural drug would rather commonly produce significantly different responses. So it is necessary to determine some crucial physicochemical characters of each sample before its pharmacological study to ensure that subsequent study would use same natural drugs. Therefore, along with the pharmacological study, the test drug was also subjected to a physicochemical study, the evaluation of their ash value, extractive value, and qualitative analysis is of great significance. Therefore, present study deals with physicochemical and phytochemical investigation of Tukhm-e-Katan consists of dried seeds of *Linum usitatissmum* Linn. It is versatile and blue flowering rabi crop belonging to linaceae family, commonly known as Flaxseed or Linseed in English (Anonymous, 2007). It is one of the most ancient crops cultivated in Egypt. It is also cultivated in India as an oil seed plant. The plant has shown diverse biological and pharmacological activities. It has been used in Unani Medicine and Traditional Systems of Medicine from time immemorial. Its seed and oil are used in various diseases such as asthma, cough, bronchitis, pleurisy, pneumonia, joint pain, renal colic, renal calculi, rheumatic swelling (Nadkarni,

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1982). In recent years, considerable research has been done on an array of plants having medicinal values. Tukhm-e-Katan is a famous Unani drug used in a number of pathological conditions. Although entire plant has medicinal value but its seed and oil are more important and have broad medicinal values. Tukhm-e-Katan is an annual herb of about 0.7 m high with blue flowers and a globular capsule. The seed are ovate, flattened and obliquely pointed at one end, about 4-6 mm long and 2-2.5 mm broad. The testa is brown, glossy and finely pitted, odorless, taste mucilaginous and oily. If cruciferous seed are present, a pungent odour and taste may develop on crushing and moistening (Evans, 2009). The Flax seed plant is native of Egypt, extensively cultivated in India, chiefly in Bengal, Bihar and the United provinces (Nadkarni, 1982), cultivated throughout of India and altitudes of 2000 meters above sea level (Chopra et al., 1958). The seed coat contain mucilage, the surface are studded with fine pits or depression with a ridge just below the apex, having the hilum in the hollow. Seed nuclei or cotyledons are two, large and oily and contained within the external covering, within which is a thin mucous envelope (Khory and Katrak, 1993), Linseed oil is fixed oil expressed from linseed. It is clear yellowish brown oil, having characteristic odour, and taste bland. Gradually, thickens on exposure to air spread in thin film, a hard transparent varnish (Anonymous, 1992). The drug, Katan possess anti-bacterial, demulcent (Evans, 2009), anti-inflammatory, expectorant, laxative, and Analgesic activity (Khory and Katrak, 1993), diuretic, emollient, emmenagogue, aphrodisiac (Kiritikar and Basu, 1995), sedative, lithotriptic, activity (Bhattacharjee, 2004), So medicinally used in Renal colic, cystitis, vesicle irritation, renal calculi, boils, piles (Khory and katrak, 1993), leprosy, burn, ulcer, asthma, gonorrhea (Kiritikar and Basu, 1995), pleurisy, pneumonia, cough, baldness, joint pain, gout (Ghani, 2010; Nadkarni, 1982). According to Unani it has Mohallil-e-waram (Nabi, 1958; Khan, 1331)' Dafe-e-Sua'al, Muqawwi-e-Bah Muqawwi-e-Aaza, Mulayyin (Nabi, 1958; IbnSena, 2007). No work has been reported regarding standardization of this drug so far. Keeping in mind the medicinal importance of this plant in Indian system of Medicine specifically Unani system of Medicine a physico-chemical and phytochemical study of Katan was carried out following various parameters.

Material and Methods

The test drugs Tukhm-e-Katan (*Linum usitatissmum* Linn.) was procured from the local market of Aligarh. And are properly identified according to the botanical, Unani and Ayurvedic literature and then confirmed in pharmacognosy section of department of Ilmul Advia. A herbarium sample of the test drugs were prepared and submitted to mawalid-e-salasa museum of the department after identification for future reference, with the voucher no of SC- 0184/15. The seed of Katan was cleaned from the earthy material, and were dried in hot air oven at 50°C for 6 hours and powdered in haawandasta (Iron mortor) as a course powder for extraction. Finally the powder was stored in air tight container for experimental study.

Figure 1. (a) Plants of *Linum usitatissimum* Linn (b) Seeds of *Linum usitatissimum* Linn.

Physicochemical Studies

The Physicochemical study include the determination of organoleptic characters,extractive values of the test drug in different solvents, alcohol and water soluble contents, moisture content, ash values, loss of weight on drying, bulk density and pH values.

Ash value determination

Total ash

About 2 to 3 gm accurately weighed powdered drug was incinerated in silica crucible (previously ignited and weighted) at a temperature not exceeding dull red heat (450°C) in muffle furnace until free from carbon. The crucible was cooled in dessicator and weighted. The percentage of total ash was calculated with reference to air dried drug (Afaq et al., 1994; Jenkins et al., 2008; Anonymous, 1968).

Water soluble ash

The ash was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on ash less filter paper (Whatmann Filter paper No.42). It was washed with hot water and was incinerated along with filter paper in a previously weighted silica crucible at a temperature not exceeding 450°C to a constant weight. The weight of the insoluble matter was subtracted from the weight of total ash and the difference in weight helps in determining the weight of the water soluble ash. The percentage of the water soluble ash was determined with reference to the air dried drug (Anonymous, 1968; Jenkins et al., 2008).

Acid insoluble ash

The ash was boiled with 25 ml of dilute Hydrochloric acid

for 5 minutes. The insoluble matter was collected on ash less filter paper (Whatmann Filter paper No.42). It was washed with hot water and the insoluble matter was incinerated along with filter paper in a previously weighted silica crucible not exceeding 450°C to a constant weight. The percentage of the acid insoluble ash was determined with reference to the air dried drug (Anonymous, 1968; Jenkins et al., 2008).

Moisture content

The moisture content of the drug was determined by Toluene distillation method (Dean and Stark Method). 10 g of test drugs was taken in the flask of toluene distillation apparatus and 75 ml of distilled toluene was added and heated for subsequently for 5 hours. The volume of the water collected in the receiver tube (graduated in ml) was noted and the percentage of moisture content was calculated (Jenkins et al., 2008; Afaq et al., 1994).

Loss of weight on drying

10 g of powdered drug was taken, spread uniformly as a thin layer in a shallow petridish. It was heated at a regulated temperature of 105ºC, cooled in a desiccator and weighted. The process was repeated many times till two consecutive weights were found constant. Loss in weight was calculated with respect to initial weight in reference of percentage (Anonymous, 1987; Afaq et al., 1994).

Determination of 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% aqueous solution

An accurately weighted 1 g of drug was dissolved in distilled water and the volume was adjusted accurately to 100 ml in a conical flask and allowed to stand overnight. It was filtered and the pH of 1% solution was measured with pH meter at particular temperature until two successive reading agree within +0.02 unit (Anonymous, 1987; Jenkins et al., 2008; Anonymous, 1968).

The pH value of 10% aqueous solution

An accurately weighted 10 gm of drug was dissolved in distilled water and the volume was adjusted accurately to 100 ml in a conical flask and allowed to stand overnight. It was filtered and the pH of 10 % solution was measured with PH meter at a particular temperature until two successive reading agree within +0.02 unit (Anonymous, 1987; Jenkins et al., 2008; Anonymous, 1968).

Bulk density

The tapped density is an increased bulk density attained after mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume readings are taken until little further volume change is observed. The mechanical tapping is achieved by raising the cylinder and allowing to it drop, under its own mass, a specified distance by either of manually methods or with the help of Apparatus. The bulk and tapped density are expressed in g/ml, here ml and cm^3 are equivalent volume (Anonymous, 2016).

$$\text{Bulk Density} = \frac{\text{Wieght of the Powder drug /g}}{\text{Volume of Cylender in cm3 or m}}$$

Determination of extractive values

The extractive values of all the test drugs in different solvent viz. Petroleum ether, diethyl ether, chloroform, ethyl acetate acetone, ethanol and distilled water were determined with the help of soxhlet apparatus (Successive method). The heat was applied for 6 hours on a heating mantle, after that it was evaporated on water bath till the weight become constant. The temperature of heating mantle and water bath was maintained according to the solvent used for the extraction. The extracts were filtered and after evaporation of the solvents, the extractive values were determined and percentage of extract was calculated with reference to the air dried drug. The procedure was repeated for three times and the mean value for each extract was calculated (Anonymous 1968; Anonymous 1987).

Water and Alcohol Soluble Contents

Five gram of the air dried powdered drug was taken with 100 ml of distilled water, in a glass Stoppard conical flask for 24 hours. The mixture was carefully shaken frequently for 6 hours and then allowed standing for 18 hours. It was filtered and 25ml of filtrate was evaporated to dryness on a water bath. The residue was dried at 105°C to constant weight, cooled in desiccator for 30 minutes and weighed. The percentage of water soluble matter was calculated with reference to the amount of air dried drug. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water (Anonymous, 1968).

Phytochemical qualitative analysis

The qualitative analysis of different chemical constituents present in test drugs was carried out according to the scheme proposed by (Bhattacharjee et al., 1969).

The powder of the test drugs was extracted with petroleum ether (BP, 60-80°C). The petroleum ether extract (I) was tested for 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, phenols, and sterols/terpenes for confirmation. The defatted mark was divided into two portion, one portion was extracted with hot water and the other with ethanol

(70%). The aqueous (IV) and ethanolic (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/terpene, whereas water soluble portion (VII) was tested for glycosides. The water soluble portion again hydrolysed with 5% hydrochloric acid and extracted with chloroform. The aglycone portion (VIII) was tested for insoluble hydrochloride of alkaloids. 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 any alkali (ammonia) and extracted with immiscible solvent (ether). The solvent soluble part (XI) was again tested for alkaloids.

1. Test for alkaloids

A drop of Dragendorff's reagent in the extract was added. The brown precipitate shows the presence of alkaloids (Afaq et al., 1994).

Hager' Test

Few drops of Hager's reagent were added in 1 ml of alcoholic test solution. The presence of yellow colour precipitate indicates the presence of alkaloids.

Wagner' Test

Few drop of Wagner' reagents were added in 1 ml of alcoholic test solution dissolved with 2 ml of dil. HCl. The presence of yellow brown colour precipitation indicates the presence of alkaloids (Afaq et al., 1994).

2. Test for carbohydrate / sugars

I - 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.

II - Molisch test

In an aqueous solution, α-napthol was added. Afterwards, concentrated sulphuric acid was gently poured. A brown colour ring at the junction of two solutions indicates the presence of the sugar, (Afaq et al., 1994).

3. Test for flavonoids

A piece of magnesium ribbon was added to the ethanolic extract of the test drug followed by drop wise addition of concentrated HC1. Colour ranging from orange pink to red is a confirmatory test for flavonoids (Fransworth et al., 1966).

4. Test for glycosides

The test solution is to be filtered and sugar is removed by fermentation with baker's yeast. The acid is removed by precipitation with magnesium oxide or barium hydroxide. The remaining alcoholic extract contains the glycosides was subsequently detected by the following methods,

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

b. The Molisch's test is done for sugar using α-napthol and concentrated sulphuric acid (Afaq et al., 1994).

5. 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 (Afaq et al., 1994).

6. Test for protein

Million's reaction

To the test solution, Million's reagent was mixed and white coloured precipitate showed the presence of proteins.

Biurette's reaction

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

Xanthoproteinic reaction

In the test solution, concentrated nitric acid was added. A yellow precipitate appeared which dissolved in strong solution of ammonia and gave yellow colour, showing the presence of proteins (Afaq et al., 1994).

7. Test for Starch

0.015 g of Iodine and 0.015 g of potassium Iodide was added in 5 ml of distilled water, 2 ml of iodine solution formed was added to 2 ml of aqueous test solution. The presence of blue colour indicates the presence of starch (Bhattacharjee et al., 1969).

8. Test for phenol

5-8 drops of 1% aqueous solution of lead acetate was added to aqueous or ethanolic test solution. The presence of yellow coloured precipitate indicates the presence of phenols (Afaq et al., 1994).

9. Test for Sterol / Terpenes

Salkowski reaction: In the test solution of chloroform, 2 ml concentrated sulphuric acid 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 sterols / terpenes (Afaq et al., 1994).

10. Test for Amino Acids

The ethanolic 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 (Afaq et al., 1994).

11. Test for Resin

The test solution was gently heated and acetic anhydride was added in it. After cooling, one drop of sulphuric acid was mixed. A purplish red colour that rapidly changed to violet indicates the presence of resins (Afaq et al., 1994).

Test for Saponins

(a) The defatted marc (0.5 gm) was boiled with water for 2 minutes in a test tube. After cooling, the mixture was vigorously shaken and then left for 3 minutes. The amount of honey comb frothing was classified as- no froth – negative; froth less than 1cm - weakly positive; froth greater than 1cm - highly positive; froth greater than 2 cm - strongly positive (Bhattacharjee et al., 1969).

(b) The marc was boiled with water for 2 minutes. After cooling haemolysis test was performed. Haemolysis of blood indicated the presence of saponins (Bhattacharjee et al., 1969).

Fluorescence analysis

(i) Fluorescence Analysis of powdered drugs

Fluorescence analysis of the powdered drugs were done for identification, the powdered drugs were treated with different chemicals and observed in day light and under ultra violet light. The changes in colour were noted.

(ii) Fluorescence Analysis of the successive extracts of the test drugs

Successive extracts of the test drugs viz. Petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol and aqueous extract were observed in day light and UV lights.

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, by taking petroleum ether: diethyl ether in 1:1 ratio and n-butanol: Acetic acid: Water in 5:1:4 ratio as the mobile phase. The Rf values of the spots were calculated by the following formula (Afaq et al., 1994; Anonymous, 1968).

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

Results and discussion

Physicochemical Studies

The physicochemical evaluation is an important parameter in detecting adulteration or improper handling of drug. The efficacy of drug mainly depends upon its physical and chemical properties, therefore, the determination of physicochemical characters and thereby the authenticity of drug is necessary before studying it for pharmacological activities. The techniques involved in the process of standardization encompass different parameter that together constitutes the profile of a drug. Determination of physicochemical properties also provides an index of purity and authenticity of the drug that in turn helps in quantifying the pharmacological effects and determination of the doses for various degrees of effects. Physicochemical study is also important because it helps in characterizing different constituents or group of constituents that frequently lead to establish the structure activity relationship and the likely mechanism of action of the drug. The percentage of different constituents also gives an idea of the magnitude and intensity of the effect of the drug. Apart from the degradation in the quality of the drugs that occurs due to climate, soil and processing condition, adulteration too contributes to its variability. Thus, the physicochemical study of the drug is a crucial aspect of the research study. The present study determines a comprehensive range of physicochemical characters of the drug according to the parameters used in pharmacopeia which may serve as the standard for ensuring optimum efficacy and safety of various

Table 1. Organoleptic characters of KATAN (*Linum usitatissimum* Linn.)

Parameters	Katan
Colour	Deep brown
Appearance	Coarse
Odour	Characteristic
Taste	Mucilaginous & oily

Table 2. Physicochemical study of Powder of Katan (*Linum usitatismum* L)

Parameters	Percentage (%)*
Total ash	3.79±0.029
Acid insoluble ash	2.91±0.014
Water soluble ash	0.96±0.012
Soluble Part	
Ethanol soluble	33.06±0.96
Aqueous soluble	12.26±0.70
Successive Extractive Values	
Pet. Ether	30.56±0.08
Di-ethyl ether	7.93±0.086
Chloroform	4.20±0.170
Ethyl. Acetate	2.90±0.20
Acetone	1.70±0.050
Alcohol	2.60±0.011
Aqueous	8.50±0.050
Moisture content	7±0.57
Loss on Drying	7.72±0.32
pH values	
1% water solution	7.62±0.01
10% water solution	6.68±0.07
Bulk density	0.73±0.00

*Note: Values are average of three experiments

Table 3. Preliminary Screening of major Phytochemical of Katan (*Linum usitatissmum* L)

Chemical constituents	Tests/reagent	Inference
Alkaloid	Dragendorff's reagent	+ve
	Hager´s test	+ve
	Mayer´s reagent	+ve
Carbohydrate	Molisch´s Test	+ve
	Fehling´s test	+ve
Glycoside	Reaction with barium hydroxide	-ve
Flavanoids	Mg ribbon and Dil. Hcl	+ve
Tannins	Ferric chloride test	-ve
Protein	Xanthoproteic test	+ve
	Biuret´s test	+ve
Sterol/Terpenes	Salkowski reaction	+ve
Amino acid	Ninhydrin solution	-ve
Resins	Acetic Anhydride Test	-ve
Phenol	Lead acetate Test	+ve
Saponin	Frothing with $NaHCO_3$	+ve

*Indications: „-ve" Absence and „+ve" presence of constituent

samples of the drug. Katan **(***Linum usitatismum* Linn.**)** has been in use since times immemorial to treat wide range of indications. Present study deals with physicochemical and phytochemical investigation of dried seed of Katan (*Linum usitatismum* Linn). The

Table 4. Fluorescence analysis of extraction of Katan (*Linum usitatismum* L.)

Extracts	Day Light	UV Long	UV Short
Pet. Ether	Orange	Purple	Bright Green
Di-ethyl ether	Light Brown	Light Green	Dark Brown
Chloroform	Brown	Green	Black
Ethyl-Acetate	Light Brown	Green	Black
Acetone	Brown	Light Green	Bluish
Alcohol	Brown	Green	Black
Aqueous	Dark brown	Dark Green	Black

physicochemical investigation of the certain medicinal plant will be helpful for evaluation of nutritive value and preparation of Unani drugs and medicine.

Phytochemical studies

Phytochemical screening help to reveal the chemical nature of the constituents of Linseed (*Linum usitatismum* Linn.) extract. Phytochemical analysis of extract showed that it contain Alkaloid, saponin, flavonoids, sterol and triterpenes, were found in the extract, and are potent water soluble anti-oxidant. Flavonoids are phenolic compound that act as primary anti-oxidents or free radical scavengers. Since these compounds were found to be present in the extract, it might be responsible for the potent anti-oxidant capacity of flax seed (Table 3). The extractive values are a

Table 5. Fluorescence analysis of powder drug Katan (*Linum usitatisimum* L.) with different chemical reagents

Powdered drug + Chemical Reagent	Day light	UV short	UV long
Powdered drug + Conc. HNO_3	Brown	Light Green	Black
Powdered drug + Conc. HCl	Brown	Light Green	Black
Powdered drug + Conc. H_2SO_4	Dark Brown	Dark Green	Reddish Black
Powdered drug + 2% Iodine solution	Dark Red	Light Green	Black
Powdered drug + Glacial Acetic Acid +HNO_3	Light Brown	Light Green	Green
Powdered drug + Glacial Acetic acid	Light Brown	Light Green	Black
Powdered drug + NaOH (10%)	Light Brown	Green	Light Green
Powdered drug + Dil. HNO_3	Light Brown	Light Green	Green
Powdered drug + Dil. H_2SO_4	Dark Brown	Dark Green	Black
Powdered drug + Dil. HCl	Light Brown	Light Green	Black
Powdered drug+ Dragendorff's	Golden	Bright Green	Black
Powdered drug + Wagner's Reagent	Light yellow	Dark Green	Black
Powdered drug + Benedict's reagent	Greenish Blue	Light Green	Dark yellow
Powdered drug + Fehling reagent	Light Brown	Light Green	Green
Powdered drug + KOH (10%) Methanol	Light Brown	Light Green	Black
Powdered drug + $CuSO_4$ (5%)	Light Blue	Light Green	Cherry Red
Powdered drug + Ninhydrin (2%) in Acetone	Brown	Green	Black
Powdered drug + Picric Acid	Yellow	Green	Dark yellow
Powdered drug + Lead Acetate (5%)	Brown	Light Green	Dark brown

parameter for detecting the adulteration in any drug. The amount of the extracts that the drugs yield in a solvent is often an approximate measure of the amount of a certain constituent present in the drug. Therefore, for establishing the standard of any drug the extractive values play a major role (Table 2). Ash value is the residue that remains after complete incineration of the drug. Ash value plays an important role in ascertaining the standard of a drug, because the dust, earthy and un-required matters are generally added for increasing the weight of a drug resulting in the higher ash percentage. Therefore, the ash value determination furnishes the basis of judging the identity and cleanliness of a drug and give information related to its adulteration with inorganic matter (Table 2). The percentage of solubility of powder drugs is also considered as an index of purity. Different percentage of alcohol varies with respect to soluble extractives, whereas, the drugs obtained from different source may produce different extractive values, extracted with the same concentration of alcohol (Table 2). Thin layer chromatography is one of the important techniques used for detecting the adulteration of the drugs. The various compounds present in the drug separate, depending on the affinity of mobile and stationary phases. The resolution of different kinds of chemical components is determined by using TLC and calculating the R_f values after detecting the spots in order to standardize the drug for its identity. If the drug is adulterated there might be appearance of the other compounds as adulterant, in turn may increase the number of spots. On the other hand the exhausted or deteriorated drugs may loose the components and the number of spots appeared might be less. R_f values of various spots appeared in different solvents system have been noted in day light, UV light and the treatment with iodine vapors (Table 6).

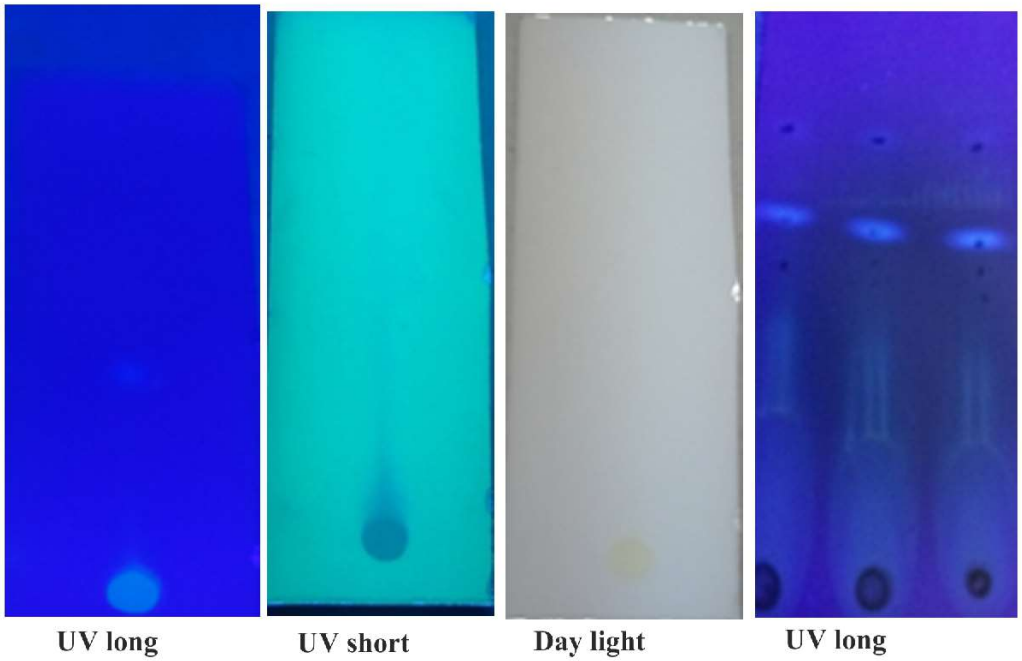

Figure 2. TLC of Petroleum ether Extract of Katan (*Linum usitatissimum* L.)

Table 6. Thin Layer Chromatography of Petroleum ether extract of Katan

Detection	Solvent System as petroleum ether : diethyl ether (2:1)	
	No of spots	Rf value and colour of spots
Day light	0	0
UV short	1	0.44 (green)
UV long	1	0.44 (light blue)
Iodine vapor	0	0
Petroleum ether extract		
UV short	4	0.46 (black), 0.52 (green), 0.56 (green), 0.66 (black)
UV long	2	0.56 (light blue), 0.66 (blue),

Conclusion

The present study has determined some crucial physicochemical characters of the test drugs by way of their standardization, so that, future studies may be carried out on samples found comparable on the basis of these characters, thereby, ensuring the reproducibility of the scientific study of these drugs.

Conflicts of interest

The authors declare there is no conflict of interests.

References

Afaq SH, Tajuddin, Siddiqui MMH. 1994. Standardization of herbal Drugs pp. 14-46. AMU. Press Aligarh.

Anonymous. 1968. British Pharmacopoeia, pp. 1208-09, 1276-77, 1285-88. General Medical Council, Pharmaceutical Press, Blumsberg Square, London.

Anonymous. 1970. Pharmacopoeia of India (Edn. 2nd); pp. 277-78, 650-51. Govt. Of India. Ministry of Health, Manager of Publication, Delhi.

Anonymous. 1992. Standardization of Single Drugs of Unani Medicine Part II, pp. 211-215, 276-281,301-306. CCRUM, New Delhi.

Anonymous. 2007. The Unani Pharmacopia of India Part. I. Vol. I. pp. 50-51. Central council for research in Unani Medicine.

Anonymous, 2016. Bulk Density and Tapped Density of powders The international Pharmacopoeia- Sixth Edition, pp. 1-4. WHO Document QAS/11.450.

Anonymous. 1987. Physicochemical Standards of Unani Formulations Part. II, pp. 274, 277. Central Council for Research of Unani Medicine, New Delhi.

Bhattacharjee AK, Das AK. 1969. Phytochemical survey of few Mysore Plants. Economic Botany, 23(3):274-276.

Bhattacharjee SK. 2004. Hand Book of Medicinal Plants 4thedn, pp. 205-206. Pointer Publishers, Jaipur.

Chopra IC, Handa KL, Kapur LD. 1958. Indigenous Drugs of India 2ndEdition, pp. 512- 513, 677. U. N. Dhur And Sons Private Limited, 15, Bankim Chatterjee Street Calcutta-12.

Evans WC. 2009. Trease and Evans Pharmacognosy 16thedn. pp. 188. Saunders Elsevier Edinburgh London New York Philadelphia St Louis Sydney Toronto.

Fransworth NR. 1966. Biological and phyto-chemical screening of Plants, Journal of Pharmaceutical Sciences, 55(3): 225-276.

Ghani MN. 2010. Khazayinul Advia. 1st Edition. Vol. II. pp. 138-142. Central council for Research In Unani Medicine, Ministry of Health and Family Welfare, Govt. of India, New Delhi.

Ibn Sina. 2007. Alqanoon Fit-Tib (Urdu translation by Golam Husain Kantoori), Vol. II, pp. 62-63. Kocha Chilan Daryi Ganj, New Delhi.

Jenkins GL, Knevel AM, Digangi FE. 2008. Quantitative pharmaceutical Chemistry, 6th edition, pp. 29, 230. CBS Publishers and Distributors Pvt. Ltd.

Khan MA. 1313H. Muheet-e-Azam part I, Vol. IV. pp. 34-35. Matbaa Nizamee. Kanpur.

Khory RN, Katarak NN. 1993. Materia Medica of India and Therapeutics, 3rd Reprint Edition. pp. 235-236. Neeraj Publishing House Delhi- 110052.

Kirtikar KR, Basu BD. 1995. Indian Medicinal Plants. Vol. I, pp. 408-410. International Book Distributers, Dehradun.

Nabi MG. 1958. Makhzan Mufradat wa Murakkabat-e-Azam. ma'roof ba Khawasul Advia pp. 46-47. (Narain Das Jangali Mal), jayyed Barqi Press, Ballimaran, Delhi.

Nadkarni KM. 1982. Indian Materia Medica. 3rd Edition. Vol. 1, pp. 743-746. Popular Book Depot Bombay 7, Dhootapapeshwar Prkaashan Ltd.