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Original Article

Physicochemical Standardization and Safety Evaluation of Khar-e-Khasak Khurd (Fruits of *Tribulus terrestris*): A Unani Drug

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ABSTRACT

Background: *Global resurgence of interest in herbal medicines has led to an increase in the demand due to their potential of being effective, safe and economic source of natural drugs. This great 'shift' of universal trend to natural selection from synthetic to herbal medicine has led to a decline in their quality, primarily due to lack of adequate regulations pertaining to drugs. They are very often procured and processed without any scientific evaluation and launched into the market without any mandatory safety and physicochemical study.* ***Methods:*** *An attempt has been made on a well-known drug of Unani medicine Khar-e-Khasak Khurd (Fruits of Tribulus terrestris) by evaluation of Physicochemical parameters (Extractive values, Solubility, Moisture content, Ash Values, pH of 1% & 10% solution, Loss of weight on drying, Bulk Density (Poured & Tapped densities), Thin Layer Chromatography & Fluorescence analysis).* ***Results:*** *Microbial load, Aflatoxins, Pesticidal residue and heavy metal analysis was also done in the sample available and it was found to be free from contaminants and safe for use.* ***Conclusion:*** *This study provides quality standards and safety profile of Khar-e-Khasak Khurd that could be matched for its uniform and safe therapeutic effects for any study to be done ahead*

Keywords: *Khar-e-Khasak Khurd, Tribulus terrestris, physicochemical standardization.*

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INTRODUCTION

Khar-e-Khasak Khurd consists of the dried fruits of *Tribulus terrestris* of family Zygophyllaceae (Anonymous, 2007). In Sanskrit it is also known as 'Gokshura' that signifies 'Cow's hoof' due to its resemblance with the cocciwhen adhering together in pairs to a cloven hoof or 'Ikshugandha' that implies to the specific aroma of the plant (Dymock *et al.,* 1890; Khory and Katrak, 1985). *Tribulus terrestris* is an annual to rarely procumbent herb upto 90 cm tall having slender fibrous and spindle shaped root; slender, prostate branched purple colour stem that bears yellow solitary axillary flowers, occurs in long raceme and blooms in July to September, peduncles shorter than leaves; leaves are opposite, pinnate compound with 4-12 obovate leaflets; one of two pair is always smaller than the other, stipules lanceolate and hairy. Fruits occur as pair as cocci, covered with stout blunt spines with faintly aromatic odour and little taste (Anonymous, 1998; Hooker, 1982). It is commonly found as weed in pasteur lands, roadsides chiefly in hot dry and sandy areas. It grows throughout India as prostrate herb and upto 3,000 meter in Kashmir (Anonymous, 2007; Anonymous, 2010). It is an age old herb that was known to Greek physicians and was in use in South Europe as aperient and diuretic (Nnadkarni, 1954).

In classical Unani texts Khar-e-Khasak is a thorny fruit of a plant that grows in rainy season. It is of two types Khurd (small) and Kalan (large) and mostly khurd variety is used for medicinal purposes (Kabiruddin, 2000). Kkhar-e-Khasak Kalan is also known as Wilayati Gokhru found in South India and sandy areas of other countries. Plants of small Gokhru are found in North West India, spread on the ground as wild variety near roadsides. Leaves are similar to gram from which mucilage oozes out in large amount at its branching site or when they are treated with water. Each fruit bears three sharp thorns also called as Dakhni Gokhru

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(Anonymous, 1987; Ghani, 2010). Root and fruits are reported to be in use in various literatures for their medicinal properties (Nadkarni, 1954; Chughtai, 1963; Dey, 1973; Khory and Katrak, 1985; Naik, 2004; Chopra, 2006). The drug has many therapeutically properties like diuretic, emmenagogue, lactagogue, lithotriptic, laxative, demulcent, divergent, refrigerant, aphrodisiac, carminative, astringent, haemostyptic, purgative, anti-inflammatory, tonic and used in various diseases i.e. vesicular calculi, urinary discharge, strangury, sexual weakness, gout, dysuria, burning micturition, cystitis. The plant contains tribuloside and astragalin glycosides along with harmane and harmine as alkaloids.

METHODS

Khar-e-Khasak Khurd (Fruits of *Tribulus terrestris*) was procured from the local market of Aligarh. The identity was confirmed with the help of literatures available and Pharmacognosy Section, Department of Ilmul Advia, Ajmal KhanTibbiya College, Aligarh Muslim University, Aligarh. The specimen of the test drug was submitted to Mawalid-e-Salasa Museum of the Department for future reference with the voucher No of SC0188/15. The powder of gum was subjected to physicochemical and phytochemical studies to determine various constants and to evaluate its safety profile as well.

Market Sample of Khar-e-Khasak Khurd (Fruits of Tribulus terrestris)

Evaluation of organoleptic characteristics

It included the evaluation of herbal drug by size, shape, colour, odour, taste and particular characteristics like touch, texture etc. (Bijauliya, *et al*., 2017) [Table 1].

Physicochemical studies

The physicochemical study included the determination of successive extractive values of the test drug in different solvents, alcohol and water soluble contents, moisture content, ash values, loss of weight on drying, pH values, crude fibre content and bulk density (Table 2).

Successive extractive values: The successive extractive values of the test drug in different organic solvents viz. petroleum ether, diethyl ether, chloroform, alcohol and distilled water were determined using a Soxhlet's apparatus. The heat was applied for six hours for each solvent on a water bath/ heating mantle. The extracts were filtered and after evaporation of the solvents; the extractive values were determined with reference to the weight of air dried drug. The procedure was repeated three times and the mean value for each extract was calculated (Anonymous, 1968)[Table 2].

Water and alcohol soluble contents: 5 gm of the air dried powdered drug was taken with 100 ml of distilled water in a glass stoppered 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 the whole filtrate was evaporated to dryness on a water bath at 105ºC 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) [Table 2].

Loss of weight on drying: 10 gm of drug was taken, spread uniformly and thin layered in a shallow petridish. It was heated at a regulated temperature of 105 ºC, 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 (Jenkins *et. al.,* 2008) [Table 2].

Moisture Content: The toluene distillation method (Dean and Stark Method) was used for the determination of moisture content. 10 gm of drug was taken in the flask and 75ml of distilled toluene was added to it. The level of toluene should be above the drug level in the flask. The drug was submerged in toluene then it was distilled for sufficient time. The volume of water collected in receiver tube (graduated in ml) was noted and the percentage of moisture was calculated with reference to the weight of the air dried drug taken for the process. This process was repeated for three times and the mean value was calculated (Jenkins *et. al*., 2008) (Table 2).

Ash Values:

Total Ash: 2 gm of drug was incinerated in a silica crucible of constant weight at a temperature not exceeding 800ºC in a muffle furnace until free from carbon, cooled and weighed, the percentage of ash was calculated by subtracting the weight of crucible from the weight of crucible + ash. The percentage of total ash was calculated with reference to the weight of drug taken (Anonymous, 1968) [Table 2].

Water Soluble Ash: The obtained ash was boiled with 25ml of distilled water for 10 min. The insoluble matter was collected in an ash less filter paper; (Whatman No. 42), washed with hot water and ignited in crucible at a temperature not more than 800 ºC, the weight of insoluble ash was subtracted from the weight of total ash, giving the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air dried drug taken (Anonymous, 1968) [Table 2].

Acid Insoluble Ash: The total ash was boiled with 25 ml of 10% hydrochloric acid for 10 min. The insoluble matter was collected on ash less filter paper (Whatman No. 42), washed with hot water and ignited in crucible at a temperature not exceeding 800ºC till constant weight. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken (Anonymous, 1968) [Table 2].

pH Value: Determination of pH was carried out with the help of a digital pH meter (model no. HI96107, HANNA Instruments). 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 powder drug was measured (Anonymous, 1968) [Table 2].

Bulk Density

Poured Bulk Density: It was determined by pouring 50 gm of drug into a graduated cylinder and the volume occupied by the drug was measured (Table2).

Tapped Bulk Density: It was determined by measuring the volume occupied by the sample of known mass (50gm) into a graduated cylinder after subjecting to prescribed amplitude and frequency of tapping over a prescribed period of time (15 minutes) using digital tapped Densitometer (Table 2). Bulk Density was calculated by the following formula:-

Bulk Density = Mass/Volume in gm/ml

Qualitative phytochemical 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 ºC). The petroleum ether extract (I) was tested for free phenols, alkaloids and sterols/terpenes. A part of this extract was saponified and 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 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/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 any alkali (ammonia) and extracted with immiscible solvent (ether). The solvent soluble part (XI) was again tested for alkaloids (Table 3).

Test for Alkaloids: A drop of Dragendroff's reagent in the extract was added. The brown precipitate showed the presence of alkaloids (Afaq *et al.*, 1994).

Hager's test: Few drops of Hager's reagent were added in 1 ml of alcoholic test solution. The presence of yellow colour precipitate indicated the presence of Alkaloids (Afaq *et al.*, 1994).

Wagner's test: Few drops of Wagner's reagent were added in 1 ml of alcoholic test solution dissolved with 2 ml of dil. HCl. The presence of yellow brown colour precipitates indicated the presence of Alkaloids (Afaq *et al*., 1994).

***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 indicated the presence of reducing sugars.

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

Test for flavonoids: A piece of Magnesium ribbon was added to the ethanolic extract of the drug followed by drop wise addition of concentrated Hcl. Colour ranging from orange pink to red was a confirmatory test for flavonoids (Fransworth, 1966).

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 or barium hydroxide. The remaining ethanolic extract containing the glycosides was subsequently detected by the following methods:

a. 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.

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

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, showed the presence of tannin (Afaq *et al*., 1994).

Test for Proteins:

Biuret test: In 1 ml of hot aqueous extract of the drug, 5-8 drops of 10 W/V solution hydroxide was added followed by 1 or 2 drops of 3 % W/V copper sulphate solution. A red or violet colour was obtained (Anonymous, 1968).

Millon's reaction: To the test solution, Millon's reagent was mixed and white coloured precipitate showed the presence of proteins (Afaq *et al.*, 1994).

Test for Starch: 0.015 gm of Iodine and 0.015 gm 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 indicated the presence of starch (Ali, 2010).

Test for Phenol

i. Ferric chloride solution was added in 2 ml of ethanolic or aqueous test solution. Blue or green colour indicated the presence of phenols (Finar, 1973).

ii. 5-8 drops of 1% aqueous solution of Lead acetate was added to aqueous or ethanolic test solution. The presence of yellow colour precipitate indicated the presence of phenols

iii. **Libermann's Test**: 2ml of ethanolic or aqueous test solution was dissolved with 0.5 ml of 70 % H_2SO_4 followed by the addition of few drops of aqueous sodium nitrite solution (0.5%). Red colour on dilution indicated the presence of phenols (Brewster and Mc Even, 1971).

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 indicated the presence of the sterols/terpenes (Afaq *et al.*, 1994).

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 gave a blue to red-violet colour that indicated the presence of amino acids (Brewster and Mc Even, 1971).

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 indicated the presence of the resins (Afaq *et al*., 1994).

Fluorescence Analysis:

Fluorescence Analysis of powdered drugs: Fluorescence analysis of the powdered drugs was done for identification. The powdered drugs were treated with different chemicals and observed in daylight and under ultra violet light. The changes in colours were noted (Nagulan and Kumar, 2016) [Table 4].

Fluorescence Analysis of the successive extracts of test drug: Successive extracts of all the drug samples viz. Petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol and aqueous extracts were observed in day light and UV light (Nagulan and Kumar, 2016) [Table 5].

Thin Layer Chromatography (TLC)

Thin layer chromatography of different extracts of the drug was carried out on T.L.C precoated aluminium plates (silica gel 60 of F254, layer thickness 0.25 mm) by taking various mobile phases. After the development of the plates, they were sprayed with different reagents and examined under day, UV lights (UV short and UV long) and Iodine vapours to detect the spots representing various constituents. The Rf values of the spots were calculated using the following formula (Anonymous, 1968, Afaq *et al*., 1994) [Table 6].

Rf value = Distance travelled by the spot / Distance travelled by the solvent

Safety studies

Heavy Metals Determination Heavy metals including Lead, Mercury, Arsenic and Cadmium were determined in the test sample using Atomic Absorption Spectroscopy (AAS).(Table 7)

Microbiological determination tests

Total viable aerobic count (TVC): For detection of the antibacterial activity of the test drug, the total viable aerobic count (TVC) of the test drug was carried out, determined, as specified in the test procedure, using following methods:

Pre-treatment of the test drug Depending on the nature of the compound sample used, it was dissolved using a suitable method and any antimicrobial property present in the sample was eliminated by dilution or neutralization. Buffered Sodium Chloride-Peptone Solution, pH 7.0 (MM1275-500G Himedia Labs, Mumbai, India) was used for dilute the test sample.

Plate Count for bacteria: 1 ml of the pre-treated test sample was added to about 15 ml of the liquefied casein-soybean digest agar in a petridish of 90 mm diameter at a temperature not exceeding 45 0C. Alternatively the test sample was spread on the surface of the solidified medium. Two dishes were prepared with the same dilution, they were inverted and incubated at 30-350C for 48-72 hrs, unless a more reliable count was obtained in a short period of time. The number of colonies so formed was counted and the results were calculated using the plates with the largest number of colonies, up to a maximum of 300.

Plate Count for fungi: 1 ml of the pre-treated test sample was added to about 15 ml of the liquefied Sabouraud glucose agar with antibiotics in a petridish of 90 mm diameter at a temperature not exceeding 450C. Alternatively the test sample was spread on the surface of the solidified medium. Two dishes were prepared with the same dilution; they were inverted and incubated at 20-250C for 5 days, unless a more reliable count was obtained in a short period of time. The number of colonies so formed was counted and the results were calculated using the plates with not more than 100 colonies (Table 8).

Aflatoxin Estimation:

The test for the determination of aflatoxins B1, B2, G1 and G2 was carried out using LC-MS/MS. 2 gm of test drug was blended at high speed with 20 ml of 60% acetonitrile/water for two minutes. The blended sample was centrifuged for ten minutes using 1600 rpm (av.), supernatant was retained and diluted with 2 ml of filtrate with 48 ml of phosphate buffered saline (PBS, pH 7.4) to give a solvent concentration of 2.5% or less; methanol/water was prepared by taking 2 ml of sample and diluted with 14 ml of PBS (pH 7.4) to give a solvent concentration of 10% or less. The sample diluent was passed through the immunoaffinity column at a flow rate of 5 ml/ min. The column was then washed by passing 20 ml of distilled water through the column at the flow rate of approximately 5 ml/ min and dried by rapidly passing air through the column. 1.5 ml of distilled water was added to the sample elute. 500 µl of sample was injected onto the LCMS-MS (LC- Perkin, MS Applied Bio System, Model No.2000, Mobile Phase). A- Water 100%, B-ACN 100%, Column oven temperature = 30, Column ZORBAX Rx c18, narrow base 2.1×150 mm - 5 micron, Flow = 0.750 ml). The aflatoxin concentration was quantified by comparing sample peak heights or areas to the total aflatoxin standard (R-Biopharm) (Lohar, 2007) [Table 9].

Pesticidal Residue Estimation The test for the assessment of specific pesticide residues like Organochloride compounds, Organophosphorous compounds and Pyrethroids compound was conducted using GC-MS/MS (Ramakrishnan *et al.,* 2015) [Table 10).

RESULTS

Organoleptic evaluation of powdered drug was carried out, listed in Table 1:

Table: 1 Organoleptic evaluation of powdered drug

Parameter	Inference
Colour	Greenish Yellow
Appearance	Coarse
Taste	Bitter
Odour	Agreeable

Table: 2 Physico-chemical study of powdered drug of Khar-e-khasak khurd

S. No.	Parameters	Percentage (w/w)*
1	**Ash value**	
	Total ash	8.34
	Acid insoluble ash	0.89
	Water soluble ash	5.81
2	**Soluble Part**	
	Ethanol soluble	23.44
	Aqueous soluble	4.13
3	**Successive Extractive Values**	
	Pet. Ether	2.35
	Di-ethyl ether	0.29
	Chloroform	0.48
	Ethyl acetate	0.70
	Acetone	0.63
	Alcohol	2.4
	Aqueous	13.45
4	**Moisture content**	5.0
5	**Loss on Drying**	6.26
6	**pH values**	
	1% water solution	6.56
	10% water solution	6.18
7	**Bulk density**	0.50

*Note: Values are average of three readings.

Table 3: Preliminary Screening of major Phytochemicals

S.No	Chemical Constituent	Tests/Reagent	Inference
1	Alkaloids	Dragendorff's test	+
		Wagner's test	+
		Hager's test	+
2	Carbohydrate	Molisch's Test	+
		Fehling's Test	+
3	Flavonoids	Mg. ribbon test	+
4	Glycosides	Baker's yeast test	+
5	Tannins	Ferric Chloride Test	+
6	Proteins	Xanthoproteic Test	+
		Biurett's Test	+
		Million's test	+
7	Starch	Iodine Test	-
8	Phenols	Lead acetate test	+
9	Sterols/Terpenes	Hosss's reaction	+
		Liebermann Burchard's Test	+
10	Amino acid	Ninhydrin test	+
11	Resin	Acetic anhydride test	+

Indications: '–' Absence and '+' presence of constituent.

Table: 4 Fluorescence Analysis of Successive extracts of Khar-e-Khasak Khurd

	Extract	Day Light	UV Long	UV Short
1	Pet. ether	Light Brown	Light Green	Black
2	Di-ethyl ether	Dark Brown	Dark Green	Dark Green
3	Chloroform	Light Brown	Light Green	Black
4	Ethyl acetate	Light Brown	Green	Dark Green
4	Acetone	Light Brown	Light Green	Black
5	Alcohol	Brown	Green	Black
6	Aqueous	Dark Brown	Dark Green	Black

Table-5 Fluorescence Analysis of powdered drug of Khar-e-khasak Khurd with different chemical reagents

S. No.	Powdered drug (P.d)	Day light	UV short	UV long
1.	P. drug + Conc. HNO_3	Orange	Light Green	Black
2.	P. drug + Conc. Hcl	Brown	Light Green	Dark Green
3.	P. drug + Conc. H_2SO_4	Dark Brown	Black	Black
4.	P. drug +2% Iodine solution	Reddish Brown	Dark Green	Black
5.	P. drug + Glacial Acetic acid + HNO_3	Blackish Red	Light Green	Black
6.	P. drug + Glacial acetic acid	Light Brown	Light Green	Black
7.	P. drug + NaOH (10%)	Light Brown	Light Green	Black
8.	P. drug +Dil. HNO_3	Reddish	Green	Black
9.	P. drug + Dil. H_2SO_4	Dark Brown	Dark Green	Green
10.	P. drug +Dil. Hcl	Light Brown	Light Green	Black
11.	P. drug + Dragendorff's	Reddish Black	Bright Green	Black
12.	P. drug + Wagner's Reagent	Brown	Green	Black
13	P. drug + Benedict's reagent	Blue	Bluish Green	Black
14	P. drug + Fehling reagent	Golden	Light Green	Black
15	P. drug + KOH (10%) Methanolic	Brown	Light Green	Black
16	P. drug + $CuSO_4$ (5%)	Brown	Green	Black
17	P. drug + Ninhydrin (2%) in Acetone	Reddish	Dark Green	Dark Green
18	P. drug + Picric Acid	Yellowish	Bright Green	Black
19	P. drug + Lead Acetate (5%)	Brown	Light Green	Black

Table-6 Thin Layer Chromatography of Khar-e-Khasak Khurd

Treatment	Mobile phase:	No of spots	Rf value and colour of spots
Petroleum Ether Extract			
UV Short	Petroleum ether : Ethyl acetate (24:1)	3	0.21 (Purple), 0.61 (Blue), 0.69 (Blue)
UV Long		3	0.24 (Purple), 0.41 (Blue), 0.53 (Purple)
Iodine Vapour		4	0.107(Yellow), 0.384 (Dark yellow), 0.523 (Brown)
Diethyl ether Extract			
UV Long	Petroleum ether : Acetone (3:1)	6	0.09 (Pink), 0.28 (Blue), 0.46 (Blue), 0.55(Pink), 0.72 (Blue), 0.78(Blue)
Chloroform Extract			
UV Short	Toulene : Ethyl acetate (8:2)	2	0.13 (Blue), 0.33 (Blue)
UV Long		6	0.15 (Red), 0.41 (Blue), 0.48(Blue), 0.58 (Blue), 0.65(Blue), 0.72 (Blue)
Alcoholic Extract			
UV Short	Chloroform: Methanol (9:1)	3	0.435 (Brown), 0.838 (Brown), 0.935 (Brown)
UV Long		3	0.435 (Blue), 0.758 (Purple), 0.935 (Blue)
Iodine Vapour		2	0.161 (Dark yellow), 0.596 (yellow)

Table-7 Heavy Metal Analysis of powdered drug of Khar-e-Khasak Khurd

Heavy Metals	Result (mg/kg)	LOQ(mg/kg)?ermissible limit (mg/kg)	
Lead (Pb)	Not detected	2.50	Not more than 10
Mercury (Hg)	Not detected	0.5	Not more than 1
Arsenic (As)	Not detected	1.25	Not more than 3
Cadmium (Cd)	Not detected	0.25	Not more than 0.3

DISCUSSION

Proper identification and standardisation is mandatory to ensure the therapeutic efficacy of herbal drugs used for health ailments. Standardisation is an essential tool to ensure identity, purity and quality of herbal drugs. Preceding parameters were used for the physicochemical study of fruits of Khar-e-Khasak Khurd (*T.terrestris*). For establishing the standards of any drug, the extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one. Percentage of Solubility is also considered as an index of purity, as alcohol can dissolve almost all substances including glycosides, resins, alkaloids etc. The ash value determination gives the amount of inorganic content of drug which determines adulteration of drug. The moisture content of the drug is variable because mostly herbal drugs are hygroscopic and excessive moisture content provides suitable medium for the growth of different type of microorganisms like bacteria and fungi which leads to deterioration of drug. The pH provides quantitative indication of the acidity and alkalinity of a solution. Qualitative phytochemical analysis was also carried out for the determination of the presence of alkaloids, flavonoids, glycosides, tannins, phenols, resins, sterols/terpenes, sugars, starch, amino acid, proteins and saponins. Thin layer chromatography is one of the important parameters used for judging the quality and purity of the drug. The resolution of different kinds of chemical components are separated by using TLC and calculating the Rf values after detecting the spots in order to standardize the drug for its identity, purity and strength.

CONCLUSION

Physicochemical constants and phytochemical constituents present in the drug varied not only from plant to plant but also among different plants of same species depending upon various factors like collection, drying, storage and different atmospheric conditions. Physicochemical and phytochemical study helped in the identification and purification of the test drug and proved that the test sample was genuine and of fruits of Khar-e-Khasak Khurd (*T. terrestris*). Further, the study revealed a set of diagnostic parameters for the specific drug which will be useful in identification and control to adulterations of the raw drug.

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