

Comparative Physicochemical Evaluation of Kharekhasak (*Tribulus terrestris* Linn.) Before and After Mudabbar Process

Abdullah Tauheed, Hamiduddin, Salma Khanam¹, Mohd Akhtar Ali, Mohammad Zaigham

Department of Ilmu Saida (Unani Pharmacy), National Institute of Unani Medicine, ¹Department of Pharmacognosy, Al-Ameen College of Pharmacy, Bengaluru, Karnataka, India

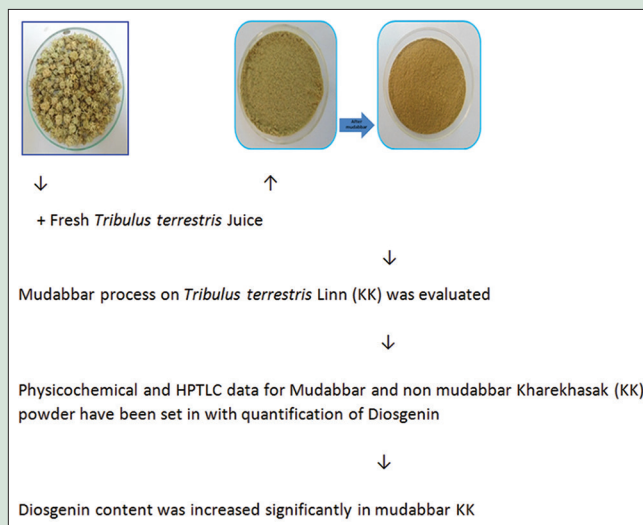
ABSTRACT

Background and Objectives: Mudabbar/Tadbeere *advia* is referred to the processes performed on the drugs to detoxify, purify, and enhance therapeutic action and to reduce its doses before making the formulations in Unani medicine. It improves quality of drugs either by optimizing its desirable characteristics or minimizing the undesirable ones; it makes drug effective, safe, and specific. There is a need of comparative evaluation to understand its significance. Tadbeer of Kharekhasak (KK) khurd (*Tribulus terrestris* Linn. fruit) is described by Rabban Al-Tabari in Firdausul Hikmat, Akbar Arzani in Qarabadeene Qadri, etc., during the compounding of aphrodisiac formulations. Mudabbar Kharekhasak (MKK) used in *Safoofe Kharekhasak* mentioned in Al-Qarabadeene was evaluated in this work. **Methods:** Mudabbar/Tadbeer process was carried out by blending fresh KK. Juice with powdered dry KK and drying it under the sun. Juice used for process is thrice the weight of dry KK powder. The KK before and after the process was evaluated using physicochemical tests: powder characterization, extractive value, alcohol and water soluble matter, ash value, loss on drying (LOD) at 105°C, pH, high-performance thin layer chromatography (HPTLC) fingerprinting, and diosgenin content. **Results:** Powder characterizations were set in. Increase in successive and nonsuccessive extractive values in various solvents, water/alcohol-soluble content, total ash, acid-insoluble ash, water-soluble ash, and sulfated ash of MKK was noted in comparison with KK. Decrease in LOD at 105°C and pH of MKK powder was observed. HPTLC fingerprinting data were developed for the identification and evaluation. Quantification of diosgenin content increased to 432.1 g/g in MKK as compared to 144.5 g/g in KK, suggesting significant increase in saponin content. **Conclusion:** Data obtained clearly indicated changes in MKK validating the classical Mudabbar process, probably to enhance/modify the action of drug. Standards for crude and MKK were established for future reference.

Key words: Kharekhasak, Mudabbar evaluation, Physicochemical, *Tribulus terrestris* Linn., Unani

SUMMARY

- Mudabbar process on *Tribulus terrestris* Linn (KK) have been validated.
- Physicochemical data for Mudabbar and non mudabbar Kharekhasak (KK) powder have been set in.
- Diosgenin content was increased significantly in mudabbar KK.



Abbreviations Used: KK: Kharekhasak, TT: *Tribulus terrestris*, MKK: mudabbar Kharekhasak, SK: Safoofe Kharekhasak, LOD: loss of weight on drying, HPTLC: High performance thin layer chromatography, BSS: British standard sieve, μ l: microliter, SEM: Standard error of mean, nm: nanometer, g: gram.

Correspondence:

Dr. Hamiduddin,
Department of Ilmu Saida (Unani Pharmacy),
National Institute of Unani Medicine, Kottigepalya,
Magadi Main Road, Bengaluru - 560 091,
Karnataka, India.
E-mail: drhamid2003@rediffmail.com
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INTRODUCTION

Mudabbar/Tadbeere *advia* is the process performed on the drugs before therapeutic use or making the formulations to detoxify, purify, and enhance their therapeutic action and to reduce their toxicity. Different processes of detoxification and purification are employed for different drugs. It improves the quality of drugs either by optimizing its desirable characteristics or minimizing the undesirable one (frequently both) to make it effective, safe, and specific. It is also done to increase the rate of absorption, to augment the effect by increasing the pharmacological effect to enhance the desirable effect, to increase the efficacy and potency, and to reduce the dose.^[1-3] Mudabbar/Tadbeer is a processing to make drugs effective and safe. Alteration in physicochemical and structural characteristics of drugs, newer actions, more specific and site-selective activity, and optimization of the efficacy, etc., are also reported. Several examples are present in Unani pharmaceuticals, in which drug action is potentiated.^[2] There is need of

evaluation of various procedures of Mudabbar on a particular drug to prove the claim and validate the process. On evaluation of effect on yield, physical, chemical, and biological parameters will be useful in standardization of the procedure as well as the product. Tadbeer (processing) used probably to potentiate the action of Kharekhasak (KK) (*Tribulus terrestris* Linn. [TT]) [Figure 1] is studied in this work for comparative evaluation.

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TT Linn. is being used in Unani system of medicine since ancient time and described by renowned Unani scholars, namely, Descorides who stated its temperament.^[2] Rhazes (865-925 AD) mentioned KK as lithotripter, aphrodisiac, and useful in strangury in his book *Kitabal Mansuri*,^[4] and *Avicenna* in *Kitab al-qanun fi al-tibb* also described it.^[5] Its important pharmacological activity is reported as aphrodisiac and spermatogenic.^[6-8] Many reports relate its aphrodisiac activity to its androgen increasing property which is due to steroidal glycoside (saponin)/protodioscin content.^[6,9] Other important activities reported are diuretic,^[7,10] antiurolithic,^[7] antiarthritic/anti-inflammatory,^[11] hepatoprotective (due to presence of tribulusamides A and B, lignanamides),^[7,12] immunomodulatory (saponins)^[13] antimicrobial activity (spirosaponins).^[14] *Tadbeer* of KK is described by Rabban Al-Tabari in *Firdausul-Hikmah*,^[15] Akbar Arzani in *Qarabadeene-Qadri*,^[16] Kabiruddin in *Al-Qarabadeene*, etc., during the compounding of aphrodisiac formulations. Mudabbar Kharekhasak (MKK) prepared by a process referred during preparation of *Safoofe Kharekhasak* (SK) mentioned in *Al-Qarabadeene* was evaluated in this work^[17] [Figure 2]. SK is a powder formulation used as an aphrodisiac.

METHODS

Physicochemical test including precompression parameter, extractive value, alcohol- and water-soluble matter, ash value, loss of weight on drying at 105°, pH value, and high-performance thin-layer chromatography (HPTLC) evaluation of the crude, as well as MKK powder with quantitative test for diosgenin content, was carried out.

Powdering and sieving of *Kharekhasak*

Crude fruits of KK were powdered using a super mixer grinder. Sieve number 80 (BSS) was used for getting fine powder.^[18]

Mudabbar process

Mudabbar process as described in *Al-Qarabadeene* was followed.^[17] Fresh KK was collected personally and subjected to hand-operated screw press to get juice; later, this juice was poured over powdered KK (passed through sieve # 80 mesh size), and the blend was dried under the sun. This process was repeated 3 times till the fresh KK. Water/juice absorbed thrice the weight of dry KK.^[17] This mass was again passed through standard sieve of # 80 mesh size and stored in air-tight containers.



Figure 1: *Kharekhasak* (*Tribulus terrestris* Linn.) fruit

Physicochemical parameters

Organoleptic properties

The properties including appearance, color, taste, and smell of powders were evaluated.^[19]

Loss of weight on drying at 105°C and ash value (acid-insoluble ash and sulfated ash) were estimated as per method mentioned in “The Unani Pharmacopeia of India, Part II.”^[20] Moisture content was estimated by toluene distillation method. Total ash, water-soluble ash, extractive values (alcohol-soluble extractive value, water-soluble extractive value) were estimated as per “Protocol for testing of Ayurvedic, Siddha, and Unani Medicines.”^[18] pH value of 1% solution and 10% solution was done by the method mentioned in “Physicochemical Standardization of Unani Formulation.” Part VI.^[19]

Successive extractive value

Ten grams powdered drug was subjected to continuous hot extraction using different solvent in increasing order of polarity successively using a Soxhlet apparatus (petroleum ether → benzene → chloroform → ethanol) for 6 h.^[21-24]

Nonsuccessive extractive values

Soxhlet apparatus was used for nonsuccessive extraction of drug. Water, ethyl alcohol, and petroleum ether were used as solvents separately for each 10 g of drug.^[24] In both successive and nonsuccessive extract value, the extracts were filtered using a filter paper (Whatman no. 1) and evaporated on water bath. Mean extractive values were determined with reference to drug taken (w/w) after repeating the process for 3 times.^[21-24]

Powder characterization

Both non-MKK and MKK powder [Figure 2] were subjected to powder characterization/precompression parameters such as bulk density, tapped density,^[25] compressibility index, Hausner's ratio,^[26,27] and angle of repose.^[27,28] Weight of powder before and after *Mudabbar* process by a digital weighing machine was also estimated.

High-performance thin layer chromatography analysis

Instrument/materials

Instruments used were CAMAG Linomat 5 (sample applicator), CAMAG TLC scanner 3, CAMAG reprostar 3, CAMAG TLC plate heater, twin trough chamber. Software used was Wincat software, Version 1.3.3. Stationary phase: Merk TLC plate, silica gel 60 F 254 (10 cm × 20 cm); mobile phase: toluene: ethyl acetate: formic acid (60:30:10) were used; and diosgenin (C01P055) was procured from Natural remedies Private Limited Bengaluru.

Development of high-performance thin layer chromatography method for standard diosgenin

HPTLC,^[29] method was developed for the estimation of diosgenin in KK and MKK^[30] TLC procedure was optimized with solvent system toluene:

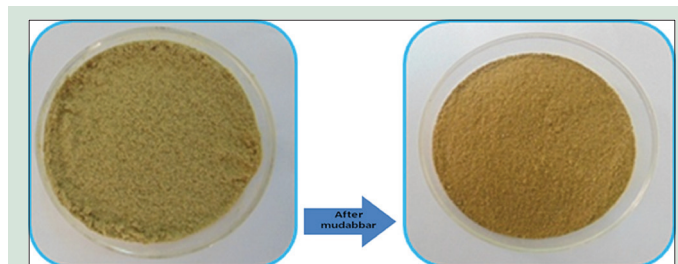


Figure 2: *Kharekhasak* powder pre- and post-*Mudabbar*

ethyl acetate:formic acid in different ratio, and the ratio which gave good resolution sharp and well-defined peak was selected. Chamber saturation time and length of development were also optimized.

Procedure

200 ng/μl of standard diosgenin was prepared in methanol. 3–17 μl of standard solution was applied as bands on the plate. The plate was developed in the saturated twin trough chamber using toluene: ethyl acetate: formic acid (60:30:10) as mobile phase. Plate was scanned in the range of 190–400 nm to determine λ max. The wavelength at which the peak showed maximum height and area was considered as λ max and was found to be 194 nm.

Linearity

Linearity was determined using eight applications of standard followed by detection of peak height and area. The linear graph of diosgenin is shown in Figure 3. HPTLC chromatogram of standard diosgenin is shown in Figures 4-6.

Quantification of diosgenin in Kharekhasak and Mudabbar Kharekhasak powder

Both extracts were subjected to acid hydrolysis (5 g of hydroalcoholic extract of processed TT [MTT] Linn and 10 g of hydroalcoholic extract of TT Linn. [KK]), extracts were dissolved in 100 ml of distilled water and 25 ml of hydrochloric acid was added, and the solution was refluxed for 1 h and extracted in 20 ml of chloroform for three times. The chloroform extract was concentrated and used as a sample. The standard diosgenin was dissolved in to give a concentration of 200 ng/μl. Standard diosgenin was applied in the concentration of 3–17 μl, and extract of plain TT and processed TT (Mudabbar) was applied in the concentration of 60 μl. Chromatograms were developed and subjected to scanning at 194 nm, and amount of diosgenin was calculated in the samples. Images of the plates were recorded using CAMAG reprostar 3. The electronic image of the chromatogram was documented in the system.

High-performance thin layer chromatography fingerprinting

Method was developed for hydro-alcoholic (50:50) extract of KK and MKK. Fingerprinting analysis was done to study the presence of active constituents of the extracts. Thirty microliters of each of samples was applied and the plate was developed and scanned at 194, 254, and 366 nm. Number of peaks and peak areas were noted. The chromatograms and data for number of peaks of sample KK at 194, 254, and 366 nm was set in the plate was dipped in vanillin sulfuric acid and photo-documented.

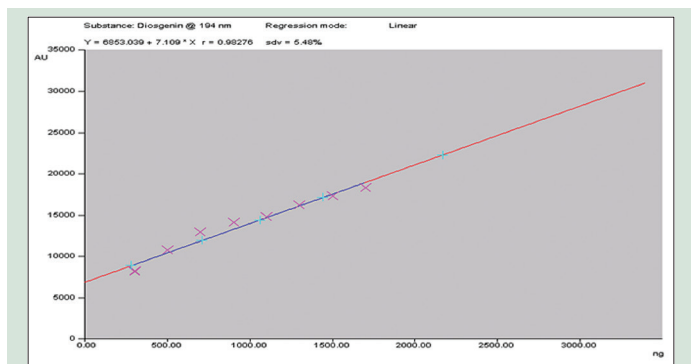


Figure 3: Quantification graph, chromatogram of diosgenin showing Linearity at 194 nm

Statistics applied

Result was analyzed by calculating mean ± standard error of mean wherever necessary.

RESULTS

Physicochemical parameters

Organoleptic properties

Appearance: powder, color: greenish yellow, taste: tasteless/very slight sweet, and smell: unrecognizable was observed in KK powder; appearance: powder, color: brownish green, taste: tasteless/slight sweet, and smell: slight unpleasant was observed in MKK [Figure 2]. Mean % age value of loss of weight on drying at 105° and moisture content by toluene distillation method, ash value (total ash, acid-insoluble ash, water-soluble ash, and sulfated ash) is, pH at 1% and 10% solution, depicted in Table 1.

Successive and nonsuccessive extractive values

The mean percentage of the extractive values of KK and MKK in petroleum ether, benzene, chloroform, and ethyl alcohol is depicted in Table 2.

Alcohol- and water-soluble matter

The mean percentage values of the water and alcohol soluble content/ extractive for KK was 9.84 ± 0.10 and 4.81 ± 0.05 and for MKK was 15.42 ± 0.11 and 6.12 ± 0.08.

Table 1: Physicochemical parameters of *Kharekhasak* and Mudabbar Kharekhasak powder

Physicochemical parameter	Mean±SEM	
	KK powder (%)	MKK powder (%)
Loss of weight on drying at 105°	5.863±0.01	4.193±0.008
Moisture content by toluene distillation method	4.666±0.33	4.333±0.33
(Ash value) total ash	10.374±0.06	11.169±0.09
Acid-insoluble ash	3.20±0.05	3.728±0.06
Water-soluble ash	2.577±0.13	3.772±0.02
Sulfated ash	9.714±0.10	10.075±0.02
pH value (%)		
1	6.41±0.005	5.736±0.003
10	5.936±0.003	4.86±0.00
Amount of diosgenin (μg/g)	144.5	432.1

SEM: Standard error of mean; KK: *Kharekhasak*; MKK: Mudabbar *Kharekhasak*

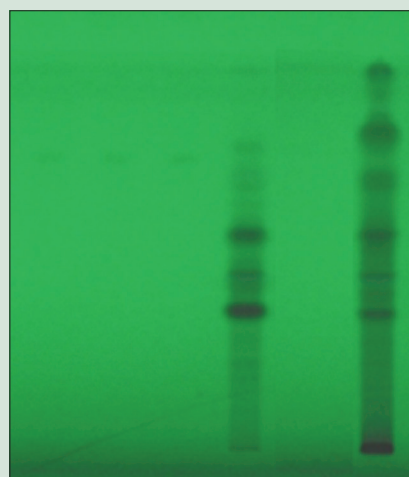


Figure 4: High-performance thin layer chromatography pattern of extracts (R→L) Mudabbar kharekhasak, kharekhasak, and standard diosgenin at 254 nm

Powder characterization

Bulk density, tapped density, compressibility index, Hausner's ratio, and angle of repose (θ) for KK and MKK are depicted in Table 3. Two hundred and fifty grams KK powder after Mudabbar process (mixed with 750 g fresh KK juice) gives 310 g MKK.

High-performance thin layer chromatography analysis

TLC procedure was optimized. The mobile phase toluene: ethyl acetate: formic acid (6:3:1) gave good resolution with R_f value 0.74. Well-defined spots were obtained, and λ max for diosgenin was found to be 194 nm. Linearity of diosgenin was found to be in the range of 600 ng to 3400 ng.

Application of validated high-performance thin layer chromatography method for quantification of diosgenin in extracts

Validated HPTLC method was applied to quantify the acid hydrolysed extracts of TT and processed TT (MKK). Peaks for TT and processed TT are shown in Figures 7-9 and photographs of HPTLC pattern are shown in Figures 4-6.

Amount of diosgenin in applied volume (60 μ l/100 μ l) is 1.441 μ g for TT (KK) and 2.166 μ g for MTT (MKK). Diosgenin content in both the extracts of TT was estimated and found to be 144.5 μ g/g in KK and 432.1 μ g/g in MKK. HPTLC pattern of extracts along with standard diosgenin is shown in Figures 4-9.

Fingerprinting of kharekhasak extracts

Number of peaks of KK samples at 194, 254, and 366 nm dissolved in methanol and chloroform is depicted in Table 4.

DISCUSSION

Color of MKK powder was little darker than the non-MKK and smell was strong which could be characteristics of an MKK. Loss on drying and moisture content were less in MKK indicating it to be less susceptible to microbial attack.^[31,32] There was increase in total ash and sulfated ash in MKK powder which shows increase of inorganic constituent in Mudabbar form. Water-soluble ash was also more in Mudabbar form clearly indicating the quality of Mudabbar powder [Table 1], decrease in pH for MKK shows that it can be absorbed more from stomach, and pH indicates better absorption site of particular drug. Acidic drug are mostly absorbed from the acidic medium in stomach and alkaline drug from intestine.^[33]

Successive extractive values and non-successive extractive values were found to be more in MKK when compared to KK. Extractive values are an indication of increase of yield of extract by Mudabbar process. Extractive value of a drug in definite solvent is an index of purity of a drug and plays a major role to determine adulteration also. Amount of the extract of a drug in a particular solvent is often an appropriate measure of the amount of a certain constituent that drug contains. The amount of drug soluble in a particular solvent is an index of its purity.^[31,32]

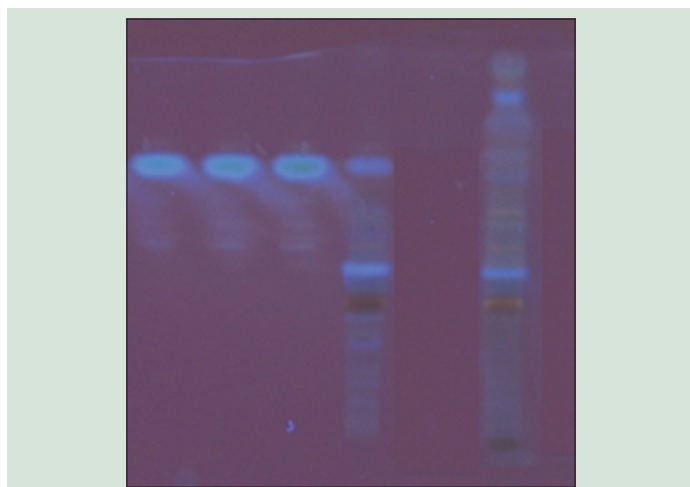


Figure 5: High-performance thin layer chromatography pattern of extracts (R→L) Mudabbar kharekhasak, kharekhasak, and standard diosgenin at 366 nm after derivatization (spraying with vanillin sulfuric acid)

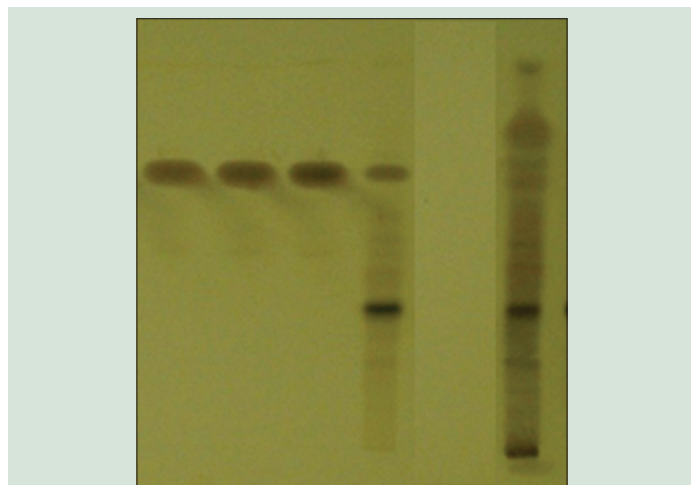


Figure 6: High-performance thin layer chromatography pattern of extracts (R→L) Mudabbar kharekhasak, kharekhasak, and standard Diosgenin at visible light after derivatization (after spraying with vanillin sulfuric acid)

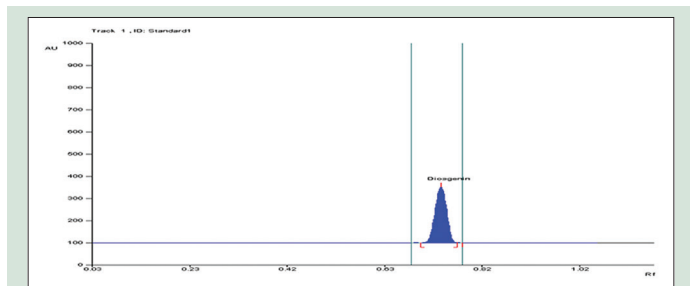


Figure 7: Densitogram of standard diosgenin at 194 nm

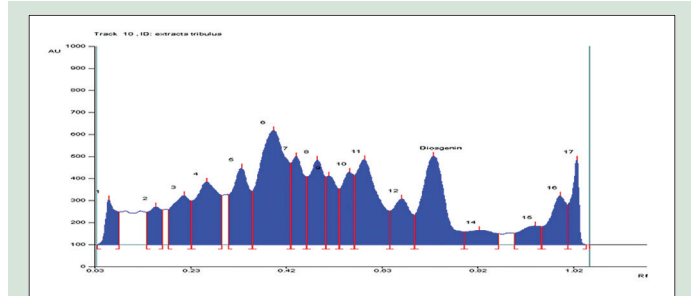


Figure 8: Densitogram of *Tribulus terrestris* (kharekhasak) at 194 nm

Table 2: Extractive values of *Kharekhasak* and Mudabbar *Kharekhasak*

Men±SEM	KK					MKK				
	Water	Petroleum ether (%)	Benzene (%)	Chloroform (%)	Ethyl alcohol (%)	Water	Petroleum ether (%)	Benzene (%)	Chloroform (%)	Ethyl alcohol (%)
Successful extractive values	-	4.283±0.07	2.903±0.03	4.366±0.14	7.296±0.20	-	4.08±0.02	2.54±0.06	5.38±0.14	8.33±0.19
Unsuccessful extractive values	13.036±0.03	3.143±0.03	-	-	6.246±0.24	19.506±0.04	4.223±0.17	-	-	9.306±0.16

KK: *Kharekhasak*; MKK: Mudabbar *Kharekhasak*; SEM: Standard error of mean

Table 3: Powder characterization of *Kharekhasak* and Mudabbar *Kharekhasak* powder

Mean±SEM (g/mL)	KK			MKK		
	Bulk density (%)	Tapped density (%)	Angle of repose	Bulk density (%)	Tapped density (%)	Angle of repose
Mean±SEM (g/mL)	0.283±0.00	0.491±0.00	50.543±0.44	0.351±0.00	0.502±0.00	41.629±0.36

Mean±SEM (g/mL)	KK			MKK		
	Compressibility index (%)	Hausner's ratio	Angle of repose	Compressibility index (%)	Hausner's ratio	Angle of repose
Mean±SEM (g/mL)	41.159±0.40	1.699±0.01	50.543±0.44	30.35±0.11	1.435±0.00	41.629±0.36

KK: *Kharekhasak*; MKK: Mudabbar *Kharekhasak*; SEM: Standard error of mean

Table 4: Number of peaks of *Kharekhasak* at 194, 254 and 366 nm

Sample	Dissolved in solvent	At wave length		
		194 nm	254 nm	366 nm
<i>Tribulus terrestris</i>	Methanol	8	10	8
	Chloroform	8	9	5

Compressibility index and Hausner's ratio measures of the tendency of powder to be compressed and indicates its flow property, which was better in MKK powder and it can provide somewhat desirable packing characteristic to it in comparison to non-MKK powder. Angle of repose was also less in MKK.^[34] These parameters also help set the characteristics of both the powder.

Hydroalcoholic extract is been used to develop HPTLC method in samples. Extracts were subjected to acid hydrolysis. Method was developed for hydroalcoholic extracts of TT (KK). 50:50 ethyl alcohol and water as a solvent was used for extraction because various study showed aphrodisiac and androgenic activity in alcoholic and aqueous extracts of drugs present in the formulation,^[35-39] and among all the solvents, 50% ethanol in water gave the highest extractable material weight.^[40] KK fruit contains steroidal saponins, teresterosin A, teresterosin E, and tribulosin which on hydrolysis give rise to steroidal saponogenin, namely, diosgenin (having pyroketon ring), gitogenin, chlorogenin, ruscogenin, and 25 D-spirosta-3,5-diene, hecogenins. Other Steroidal saponin includes gitonin, protodioscin, tribulosaponins A and B, tribulosin, and teresterosins A-K,^[7,41] considering that this HPTLC study was done and a fingerprinting was set in for future work including estimation of diosgenin which is a nonsugar component of saponin present in the formulation obtained after hydrolysis. Diosgenin, a saponin from TT, was taken as a reference standard which is an essential component.

HPTLC method developed for quantification of diosgenin reveals 144.5 µg/g diosgenin in TT (KK) and 432.1 µg/g in processed TT (MKK). These findings reflect that Mudabbar process has increases the saponin content by near about three-fold. HPTLC fingerprinting analysis data for KK and MKK powder at 194, 254, and 366 nm were also set in for future work including estimation of diosgenin. Number of spot and R_f value of each spot in a particular mobile phase is an index of purity, quality of a drug and plays a most important role to find out adulteration in drug. More froth formation was observed in MKK during acid hydrolysis,

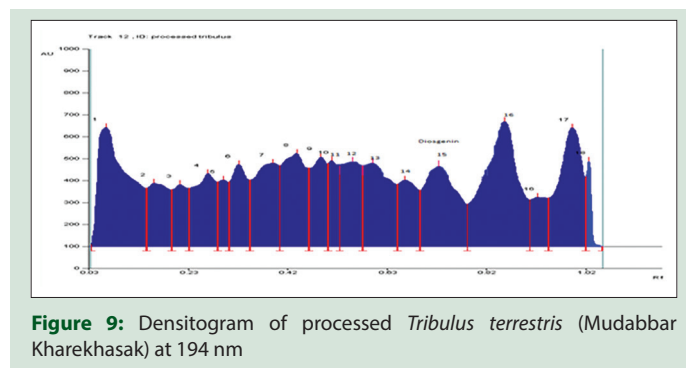


Figure 9: Densitogram of processed *Tribulus terrestris* (Mudabbar *Kharekhasak*) at 194 nm

which may be suggestive of more diosgenin content as validated through HPTLC quantification. In the present era, TT is making mark in traditional drug marketing due to its high saponin content. Evaluated process of Mudabbar may be acknowledged in the present time as it does not include any sophisticated mechanism or appliances in its processing which may make it financially more approachable. HPTLC pattern and chromatogram show some peaks extra in MKK which might have been degraded in TT (KK) alone because of drying. This increase in constituent in Mudabbar *tribulus* (MKK) may be due to addition of thrice amount of fresh juice of KK to dry TT and dry it simultaneously in the sun; this added fresh juice may also contain some other part of TT plant apart from spiny fruit alone.

Tadbeer of KK as described by Rabban Al-Tabari in Firdausul-Hikmat,^[15] Akbar Arzani in Qarabadeene-Qadri,^[16] Kabiruddin in Al-Qarabadeene,^[17] etc., during the compounding of aphrodisiac formulation was taken up for the study which includes Mudabbar process by fresh KK juice with slight variations. There are other methods of Mudabbar mentioned in the Unani text which involves process of dipping KK in cow or buffalo milk and drying it.^[2,42] These other methods of Mudabbar need investigation for the physicochemical change and pharmacological activity in KK. A significant increase of saponin content in MKK can suggest probable role of adopted *Tadbeer* process in its androgenic^[6,9] and other pharmacological effects correlated to its saponin content. These findings establish the benefit of Mudabbar process as claimed by early

Unani physicians and further validates Mudabbar process mentioned in the classical text of Unani medicine and highlighted its utility. Further study on processed KK in respect of activity evaluation is needed in view of the present findings.

CONCLUSION

Physicochemical standards (loss of weight on drying, pH, total ash, water-soluble, acid-insoluble, and sulfated ash, extractive values, HPTLC), of crude as well as MKK were established, which may be helpful for future reference. Significant increase in saponin (diosgenin) content and other reported findings suggest probably potentiating and action modification effect of Mudabbar process.

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Conflicts of interest

There are no conflicts of interest

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