ORIGINAL ARTICLE

# Formulation, physicochemical, and mating behavior evaluation of tablet modified from *Safoofe kharekhasak*: A Unani Pharmacopoeia aphrodisiac powder

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## Abstract

**Introduction:** *Safoofe kharekhasak* (SK), a powder used as an aphrodisiac in Unani Medicine. Its ingredients are *Anacyclus pyrethrum* DC. root, *Zingiber officinale* Rosc. rhizome, and *Tribulus terrestris* Linn fruit and sugar. The present study is attempted to reduce its bulkiness by converting it into extract tablets, to make it more palatable, portable, sugar-free, and to improve patient compliance. Physiochemical and aphrodisiac activity evaluation of the formulated tablet and SK was performed to validate its action. **Materials and Methods:** Tablet batch was selected prepared from the authenticated ingredient and excipients on pre- and post-compression evaluation. Physicochemical evaluation with high-performance thin-layer chromatography (HPTLC) fingerprinting with quantitative estimation of diosgenin and screening of mating behavior in rats with parameters mounting frequency (MF), intromission frequency (IF), mounting latency (ML), intromission latency (IL), ejaculatory latency in first series, and post-ejaculatory interval was carried out for the optimised batch of the tablet and SK. **Results:** Selected batch of tablet containing extract 400 mg (50%), microcrystalline cellulose, starch and lactose 124 mg (15.5%) each, SSG 8 mg (1%), Aerosil-200 16 mg (2%), and magnesium stearate 4 mg (0.5%). Hardness in Kg, friability (%), and disintegration time (in min) were 11.0 ± 0.00, 0.279 ± 0.02, and 7.906 ± 0.169, respectively. Standards for loss of weight on drying, pH, ash value, extractive values, qualitative test for various functional groups, and HPTLC fingerprinting were set in. Diosgenin content in tablet was estimated to be 63.85 µg/gm. Mating behavior study revealed significant aphrodisiac effect of the formulated tablet in the dose (140 mg/kg body weight) as per MF and EL with respect to control. **Conclusions:** Extract tablet was formulated with excipients in compliance with the analytical specification; its physicochemical standards were established. Mating behavior in rats showed potential aphrodisiac effect in rats at a specified dose.

**Key words:** Aphrodisiac, mating behavior, physicochemical, *Safoofe kharkhasak*, tablet, Unani

## INTRODUCTION

One of the unique features of Unani/Greeko-Arab medicine is that it possesses a large number of single drugs as well as formulations used as Muqawwie-Bah (Aphrodisiac), indicated in loss of libido, impotency, erectile dysfunction, etc. These drugs have been used since long time in Unani system of medicine, and sufficient claims are available showing their use by the ancient Greek and Arab physicians, for example, Hippocrates (460 BC), Dioscorides (70 AD), Razi (926 AD), and Ibne-Sina (1038 AD).[1] Infertility is a big social, biological, clinical, and personal problem among males and contributes 50% to its cause.[2] Male sexual dysfunction includes erectile dysfunction and premature ejaculation is the most common problem that contributes to infertility, distress, relationship problems, deterioration of self-image, and quality of life.[3,4] *Safoofe kharekhasak* (SK) is one such powder dosage form

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containing *Kharekhasak* or Hasak (*Tribulus terrestris* Linn) [TT] fruit (Mudabbar/processed), Sonth (*Zingiber officinale* Rosc.) dried rhizome, and Aqarqarha (*Anacyclus pyrethrum* DC.) root, and sugar in equal quantity of total drug Safoof (powder). It is described very effective for Taqwiyate Bah (as an aphrodisiac). Dose of SK described is 14 g.[5]

The powder dose is influenced by size of measuring spoon, density of powder, humidity, degree of settling, fluffiness due to agitation, etc. Overdosing and underdosing can make difference in efficacy.[6] Tablets can triumph over drawbacks related to powder as they are unit dosage forms. It is lighter, compact, easy to pack, carry and dispense as well as unpleasant odor and taste can also be masked. Microbial, physical, and chemical stability are enhanced, and climate impact is mild on tablets. Tablet provides greatest capabilities all over other dosage form.[6-8]

SK appears very effective formulation as per classical claim and contemporary research report of its ingredients, aphrodisiac activity is evident, namely, *Kharkhasak* TT is described as *Muqawwie Bah* (Aphrodisiac)[9,10] anabolic, pro-erectile, and aphrodisiac.[11] Aaqarqarha (*A. pyrethrum* DC.) is described as *Muqawwie Bah* (aphrodisiac)[9] when used orally and topically, spermatogenic and improve sexual potential;[12] and dry rhizome of Zanjabeel (*Z. officinale* Rosc.) as *Muqawwie Bah* (aphrodisiac),[9] spermatogenic[13] and having serum testosterone level increasing activity.[14,15] SK has several drawbacks for its use as a powder dosage form. It has pungent and sandy taste, leaving a singular tingling sensation in the mouth and throat due to its ingredient, Aqarqarha.[9]

It is bulky, hygroscopic with very large dose which makes it difficult to ingest. It has high sugar content. There is a need of exclusion of sugar for prescription in cases of restricted sugar intake as incidence of diabetes is increasing in the world and testicular dysfunction, impotence, decreased fertility potential and retrograde ejaculations are conditions that have been described in diabetic males, and it is also the most common cause of erectile dysfunction in men.[16] Hence, keeping in mind all the above mention facts and necessities, formulation of extract tablet of SK was attempted to improve its compliance, palatability, and effect and to assess the efficacy of modified dosage form and classical powder. Objectives of the study were formulation of extract tablets from ingredients of "SK," physicochemical, and aphrodisiac activity evaluation of tablet dosage form made from extracts of the ingredients of "SK."

## MATERIALS AND METHODS

### Procurement of Raw Drugs and their Identification

Fresh TT required for Mudabber process was collected from nearby areas of Bengaluru. Dry *Kharekhasak* fruit, Zanjabeel (dry rhizome), and Aqarqarha (root) were procured from reputed shops in Bengaluru and were identified and authenticated by the pharmacognosist, Foundation for Revitalization of Local Health Traditions (FRLHT), Bengaluru (accession number: 2968 and 2969, respectively). A specimen of drug was deposited in the drug museum of National Institute of Unani Medicine, Bengaluru, with voucher specimen no. 33/IS/Res./2015, for future reference.

### Chemicals, Solvents, and Excipient

All chemicals and the reagents used in the study were of analytical grade, HPLC grade water was used. Excipients of reputed companies were used.

### Preparation of Classical Formulation (SK)

Standard manufacturing procedure was followed for manufacturing of SK Safoof (Powder) and was prepared as per Unani classical text. Its ingredients are *Kharekhasak* (*Mudabber*) (Fruit), 1 Part, Zanjabeel (Dry Rhizome) ¼ of *Kharekhasak*, Aqarqarha (Root) ¼ of *Kharkhasak*, and Sugar in equal to weight of all the three ingredients[5] Sieve # no. 80 (Sieve Oswal, BSS), which were used for getting fine powder (described size of powder preparation as per Unani Pharmacopoeia of India).[10] Mudabber process as described in Al Qarabadeen was followed. Fresh *Kharekhasak* was subjected to hand operated screw press to get juice, later this juice was poured over powdered *Kharekhasak* (Passed through sieve no. 80), and the blend is dried under the sun. This process was repeated 3 times until the fresh *Kharekhasak* water/juice absorbed is thrice the weight of dry *Kharekhasak.* This mass is again passed through standard sieve of number 80. All the above ingredients in prescribed ratio were mixed well by applying tumbling movement and stored in airtight glass containers.

### Preparation of Tablet from Extracts of Ingredients of SK

#### *Extraction and drying*

All crude drugs were crushed with iron mortar and pestle and subjected to super mixer grinder to get coarse powder. These coarse powders of each drug were subjected to continuous hot Soxhlet extraction separately for 8 h by drug solvent ratio, 1:8[17] with hydroalcoholic solvent (ethanol and distilled water) in 50:50 ratio. The liquid extract obtained was further concentrated on rotary evaporator maintaining 50–60°C, this concentrated extract was spread on stainless steel tray and dried completely in hot air oven at 50°C, dried extract was scraped out and stored separately in airtight glass jar with silica gel pouch as a desiccant. Initially, few batches were subjected to compression directly without making granules with appropriate excipient.

#### *Wet granulation process*

Quantity of extract of each drug was maintained as per yield determined from their extractive value in the hydroalcoholic

extract in ratio 50:50 in accordance with the proportion of each drug in SK excluding the quantity of sugar [Table 1].

Different batches were prepared with different excipients in different proportions. Each excipient was weighed accurately on digital weighing balance. Extracts of all three drugs were mixed together in calculated proportion by geometrical method with the help of porcelain mortar and pestle, after sifting them with Sieve no.100. Moreover, this mixed extract (ME) was referred to as a combined extract. Microcrystalline cellulose (MCC), lactose monohydrate, sodium starch glycolate (SSG), maltodextrin, dibasic calcium phosphate, gum acacia (GA), carboxymethyl cellulose (CMC), and starch were mixed to combined extract directly with the help of porcelain mortar and pestle whenever needed according to the batch to batch variation (trial and error). After mixing they were shifted immediately to airtight glass jar, and continuous tumbling movement was produced manually for even mixing. For wetting distilled water was added to the mixture (combined extract and excipients) dropwise with an approximate estimation of one drop per gram of extract, and mixture was then blended manually until damp mass was obtained. In batches prepared by making paste of starch distilled water was added to starch separately with an approximate estimation of one drop per gram of extract to be blended, and then this mass was added to the mixture of combined extract and excipients manually. For granulation after getting damp mass, it was passed through Sieve No. 16, and wet granules were collected in stainless steel tray. The size of the granulator screen (mesh size) was decided as per diameter of punches of tablet presses.

**Figure 1:** *Safoofe kharekhasak* (Powder) and formulated tablet

Granules prepared were dried in a hot air-drying oven (Tray Drier) Pharmac, mod. No. 24 on 50°C for 30 min, and they were stored in airtight container after cooling in desiccators supplied with silica gel as desiccant. Aerosil-200 as adsorbent was first added to granules putting it in airtight glass jar and was given tumbling movement for 12 min, then afterward magnesium stearate as glidant was added in calculated proportion to cooled and dried granules in airtight glass jar and tumbling movement was produced for 4 min. Dried granules with glidant were subjected to compression by multi-station rotary presses/tableting machine (20 stations single rotary tableting machine, Cemach Machinery Ltd. Mod. No. CM-D-20-GMP Model was used), at 6 Tons pressure for all batches.

Different batches were prepared with different diluents: Lactose, MCC, starch; adsorbent: Aerosil 200 ($SiO_2$); superdisintegrant: SSG; binder: GA, CMC, and glidant: magnesium stearate with variation in their quantities summarized in Table 2. Time and temperature for drying of granules were kept constant, i.e., 30 min and 50°C, respectively. Direct compression was also tried and three batches were prepared. A total of 15 batches of extract tablets were prepared by wet granulation and from these entire batches ideal batch was selected on the basis of ideal pre-compression and post-compression parameter specifications [Table 2 and Figure 1].

### *Pre-compression parameters*

Pre-compression parameters carried out included bulk density, tapped density, compressibility index, Hausner's ratio[18] angle of repose,[19] and friability test: Friability testing apparatus Roche's friabilator (Lab India mod. no. 1020) was used for determination of friability of tablet. The procedure was repeated 3 times for mean friability value.[20,21]

### *Tablet hardness test*

Three tablets were picked up randomly, and they were individually tested for the hardness with the help of Monsanto Hardness tester (Shital Scientific Industries Sr. No. 11012010) in terms of kg/cm. The hardness of 4 kg is considered to be minimum for a tablet, i.e., satisfactorily hard.[20,21]

**Table 1:** Calculation of extract dose in human with reference to the number of drugs in classical formulation (*Safoofe kharekhasak*)

| S.NO. | Drugs | Human dose of *Safoofe kharekhasak* (g) | Extractive values 50:50 hydroalcoholic solvent (%) | Amount of extract of drug per human dose with reference to extractive value (mg) |
|---|---|---|---|---|
| 1. | *Tribulus terrestris* | 4.668 | 19.64 | 4.668×19.64÷100=0.9166 g or 916.6 mg |
| 2. | *Anacyclus pyrethrum* | 1.167 | 15.28 | 178.2 mg |
| 3. | *Zingiber officinale* | 1.167 | 8.83 | 103.02 mg |
| 4. | Sugar | 7 | ------- | Not included in extract tablet |
| Total | | 14 | | 1197.82 mg |

**Table 2:** Formulation chart of tablet batches prepared by wet granulation with post-compression parameters

| Batch No. | | Extr-act | MCC | Sta-rch | Lac-tose | SSG | $SiO_2$ | MSt. | MD | CMC | GA | Hardness (Kg) | Friability (%) | Disintegration time (Min) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Mg | 400 | 75 | 147 | 147 | 15 | 8 | 8 | - | - | - | 14 | 0.27 | 9:56 |
| | % | 50 | 9.37 | 18.37 | 18.37 | 1.87 | 1 | 1 | - | - | - | | | |
| 2. | Mg | 400 | 100 | - | 270 | 14 | 8 | 8 | - | - | - | 14 | 0.13 | 10:37 |
| | % | 50 | 12.5 | - | 33.75 | 1.75 | 1 | 1 | - | - | - | | | |
| 3. | Mg | 400 | 120 | - | 250 | 14 | 8 | 8 | - | - | - | 6 | 0.16 | 12:16 |
| | % | 50 | 15 | - | 31.25 | 1.75 | 1 | 1 | - | - | - | | | |
| 4. | Mg | 400 | 100 | - | 250 | 18 | - | 8 | - | - | 24 | 7 | 0.47 | 9:23 |
| | % | 50 | 12.5 | - | 31.5 | 2.25 | - | 1 | - | - | 3 | | | |
| 5. | Mg | 400 | 130.6 | 130.6 | 130.6 | - | - | 8 | - | - | - | 10 | 0.13 | 10:02 |
| | % | 50 | 16.66 | 16.66 | 16.66 | - | - | 1 | - | - | - | | | |
| 6. | Mg | 400 | 248 | 120 | - | - | - | 8 | - | 24 | - | 7.5 | 0.31 | 27:03 |
| | % | 50 | 31 | 15 | - | - | - | 1 | - | 3 | - | | | |
| 7. | Mg | 400 | 98 | - | 260 | 18 | - | 8 | 16 | - | - | 13 | 0.21 | 12:50 |
| | % | 50 | 12.25 | - | 32.5 | 2.25 | - | 1 | 2 | - | - | | | |
| 8. | Mg | 400 | 76 | 150 | 150 | - | - | 8 | 16 | - | - | 11 | 0.30 | 8:04 |
| | % | 50 | 9.50 | 18.75 | 18.75 | - | - | 1 | 2 | - | - | | | |
| 9. | Mg | 400 | 125 | - | 255 | 16 | - | 8 | 16 | - | - | 13 | 0.21 | 12:50 |
| | % | 50 | 15.62 | - | 31.87 | 2 | - | 1 | 2 | - | - | | | |
| 10. | Mg | 400 | 125.3 | 125.3 | 125.3 | - | - | 8 | 16 | - | - | 12 | 0.12 | 9:30 |
| | % | 50 | 15.66 | 15.66 | 15.66 | - | - | 1 | 2 | - | - | | | |
| 11. | Mg | 400 | 250 | 126 | - | - | - | 8 | 16 | - | - | 12 | 0.23 | 14:40 |
| | % | 50 | 31.25 | 15.75 | - | - | - | 1 | 2 | - | - | | | |
| 12. | Mg | 400 | 125.3 | 125.3 | 125.3 | - | 16 | 8 | - | - | - | 13 | 0.04 | 18:43 |
| | % | 50 | 15.66 | 15.66 | 15.66 | - | 2 | 1 | - | - | - | | | |
| 13. | Mg | 400 | 126.6 | 126.6 | 126.6 | - | 16 | 4 | - | - | - | 12 | 0.13 | 9:04 |
| | % | 50 | 15.83 | 15.83 | 15.83 | - | 2 | 0.5 | - | - | - | | | |
| 14. | Mg | 400 | 122.6 | 122.6 | 122.6 | 8 | 16 | 8 | - | - | - | 13 | 0.12 | 9:55 |
| | % | 50 | 15.33 | 15.33 | 15.33 | 1 | 2 | 1 | - | - | - | | | |
| 15.* | Mg | 400 | 124 | 124 | 124 | 8 | 16 | 4 | - | - | - | 11 | 0.279 | 7:9 |
| | % | 50 | 15.5 | 15.5 | 15.5 | 1 | 2 | 0.5 | - | - | - | | | |

Final selected batch*. MCC: Microcrystalline cellulose, SSG: Sodium starch glycolate, GA: Gum acacia, CMC: Carboxymethyl cellulose, MSt.: Magnesium stearate

### *Disintegration test*

Disintegration testing apparatus (Thermonik: TAB Machine Mod. no. TD 20S) was used for the determination of disintegration time.[21]

### *Uniformity of diameter*

Three tablets were picked up randomly to perform test for the determination of uniformity of diameter of tablets. Vernier caliper (UTTAR, IME type 6 inch/15 cm) was used, and diameter of each tablet was measured individually, and reading is expressed in mm.[9]

### *Weight variation*

Randomly selected 20 tablets from the final batch were weighed individually, and average weight was calculated. Individual weights were compared to average weight. Not more than 2 tablets should fall outside the permissible percentage difference range given by USP (The weight variation tolerance for uncoated tablets according to USP).[21]

## Physicochemical Parameters[22]

### *Organoleptic properties*

Appearance, color, taste, and smell of all three powders were evaluated; loss of weight on drying at 105°C: hot air oven (Labline mod. no. HO 6.7) was used;[23] Ash value-Total ash: muffle furnace (Optic technology Sr. No. 3163) was used.[24] Acid insoluble ash,[23] water-soluble ash,[24] sulfated ash;[23] and pH value: pH value of 1% and 10% solution: pH tutor (Eutech instrument Sr. no. 1544421) were used.[22]

### *Extractive values*

Alcohol soluble extractive value and water-soluble extractive value were done as per protocol for testing of ASU Medicine[24] (Shaker Labline Model No. SPL, Sr. no.10C-1187 was used) successive extractive value: With the help of Soxhlet apparatus drug was subjected to continuous hot extraction using different solvent in increasing order of polarity successively that is Petroleum ether → benzene → chloroform → ethanol. 10 g powdered was subjected to extraction with each solvent for 6 h successively;[25] non-successive extractive value: Soxhlet apparatus was used for non-successive extraction of drug. Water, ethyl alcohol, and petroleum ether were used as solvents separately each for 10 g of drug.[24] In both successive and non-successive extractive value extracts were filtered using 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.[24,25]

### *High-performance thin-layer chromatography (HPTLC) analysis[25,26]*

HPTLC method was developed for the estimation of standard diosgenin and fingerprinting in all the samples. Instrumentation used was CAMAG Linomat 5 (sample applicator), CAMAG TLC scanner 3, CAMAG Reprostar 3 (documentation instrument), and CAMAG TLC plate heater, band length: 6 mm; derivatizing agent: Vanillin sulfuric acid; twin trough chamber, dip tank, development distance: 80 mm; development time: 20 min; tank saturation time:10 min.; λ max:194 nm; WinCat software (version 1.3.3), stationary phase-Merk thin-layer chromatography (TLC) plate, Silica gel 60 F 254 (10 × 20 cm), and mobile phase-toluene:ethyl acetate:formic acid (60:30:10) were used. Diosgenin (C01P055) was procured from Natural Remedies Private Limited.

### *Quantification of diosgenin in extracts and tablet*

HPTLC method for estimation of standard diosgenin was developed and validated. TLC procedure was first optimized using pre-coated Silica Gel G 254 plates and toluene:ethyl acetate:formic acid (6:3:1) as a mobile phase which gave good resolution with Rf value 0.74. Well-defined spots were obtained, λ max for diosgenin was found to be 194 nm. The linearity of diosgenin was found to be in the range of 600–3400 ng. The validated HPTLC method was applied to quantify the hydroalcoholic (50:50) extract of TT, ME (TT, *A. pyrethrum* DC, and *Z. officinale* Rosc.) in the same ratio as they are present in extract tablet and the tablets. The extracts and the tablets were hydrolyzed to liberate diosgenin.

### *Acid hydrolysis*

Five grams of the mixture of extracts in same proportion present in tablet extract and tablet as whole were dissolved in 100 ml of distilled water and refluxed with 25 ml of hydrochloric acid 1 h and in 20 ml of chloroform for 3 times.

### *Procedure for quantification of diosgenin*

Standard diosgenin, hydrolyzed extracts, and tablet extracts were applied in the linearity range, and chromatograms were developed. Scanning was carried out 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.

### *HPTLC fingerprinting*

Fingerprinting method was developed for three individual extracts, mixture of extracts, and tablet extracts to study the presence of constituents. Procedure: 30 µl of each of the samples was applied, and the chromatogram developed. The plate was scanned at 194 nm, 254 nm, and 366 nm.

### *Test for qualitative analysis of chemical constituent of SK*

Extract was tested for glycoside, alkaloids (Dragendroff's test), test for phenols (ferric chloride test), protein (Millon's test), carbohydrates (Fehling's test), steroids (Salkowski reaction), and resins using the methods mentioned in Physicochemical

Standardization of Unani Formulation Part I.[27] The extracts were also tested for fixed and resinous oils, tannin, terpenoids, saponins, flavonoids, and reducing sugar.[28]

### Evaluation of Mating Behavior in Experimental Animal

Sixty rats (30 male and 30 female) of 3 months of age and weighing 200–250 g were obtained from Animal House Facility of National Institute of Unani Medicine (NIUM). The rats were housed in individual cages at room temperature under 23 ± 2°C and 55 =/− 5% humidity kept on 12 h light and 12 h dark cycles. The rats kept on laboratory chow and tap water *ad libitum*.

#### *Method*

The tests for mating behavior were carried out by the method of Dewsbury and Davis and Szechtman *et al.* modified by Amin *et al*.[29-33] Institutional Animal Ethical Committee (IAEC) approval was taken for the work with No. IAEC/11/08/IS. Thirty male Wistar rats of 3 months of age that showed brisk sexual activity were selected for the study. They were divided into five groups of six animals each. The animals were kept singly in separate cages during the test. The dose was extrapolated from the human clinical dose,[34] as stated in Unani literature.

#### *Dosage of drugs*

The animals in Group I (control group) were administered distilled water 1 ml; the Group II (test group) were administered the classical formulation (SK) 326.66 mg/kg body weight (BW); Group III (test group) were administered test drug 140 mg/kg BW of rat; the Group IV (test group with half dose of Group III) were administered the test drug 70 mg/kg BW; and the Group V were administered 280 mg/kg BW. All the drugs were given orally after suspending it in 1 ml distilled water. The male animals in Groups I, II, III, IV, and V were treated daily at 6:00 pm for 7 days and the animals were tested for mating behavior at 8:00 pm on the 7$^{th}$ day. The animals were brought daily in the room where they were to be tested, at the stipulated time of testing and exposed to the dim light that was to be maintained during the test. In this way, the animals were habituated to the testing conditions. The female rats were artificially brought to heat for eliciting mating behavior by the methods of Szechtman *et al.* modified by Amin *et al*.[31,32] They were administered ethinylestradiol in a dose of 100 µg, orally 48 h before the test and they were also injected subcutaneously 1 mg/animal of progesterone after dissolving it into olive oil[35] 6 h before the test. The receptivity of the female was confirmed before the test by exposing it to male rats, other than the test and control animals; the most receptive females were used for the test. The test for mating behavior was carried out on the 7$^{th}$ day after commencement of the treatment of the male animals. The test was carried out at 8:00 pm in the same room and under the light of the same intensity. The receptive female animals were introduced in the cages of male animals with one female to one male. The observation for mating behavior was immediately commenced and continued for the first and second series of mating. The test was terminated if the male failed to evince sexual interest. If the female did not show receptivity, it was replaced by another artificially warmed female. The video of experiment was shot so as to record various mating behaviors in the test and control animals. The video was later on played with the same speed and the following parameters were noted down:[35]

Mounting frequency (MF): The number of mounts before ejaculation, intromission frequency (IF): The number of intromission before ejaculation, mounting latency (ML): The time from the introduction of the female into the cage of the male up to the first mount by the male, and intromission latency (IL): The time from the introduction of the female into the cage of the male up to the first intromission by the male. Ejaculatory Latency in First Series: The time from the first intromission of a series up to the ejaculation, post-ejaculatory interval (PEI): The time from the first ejaculation up to the next intromission by the male.

### Statistical Analysis

The values of the observed parameters of the test and control animals were statically analyzed by ANOVA with *post hoc t*-test

## OBSERVATION AND RESULTS

### Identification

Raw drugs procured for the preparation of formulations (extract tablet from SK) were identified as *Kharekhasak khurd* (TT), Aqarqarha (*A. pyrethrum* DC), and Sonth (*Z. officinale* Rosc.) from FRLHT, Bengaluru.

### Formulation of Extract Tablet

#### *Pre-compression parameter*

Prepared extract granules were subjected to pre-compression parameters. The mean values of bulk density and tapped

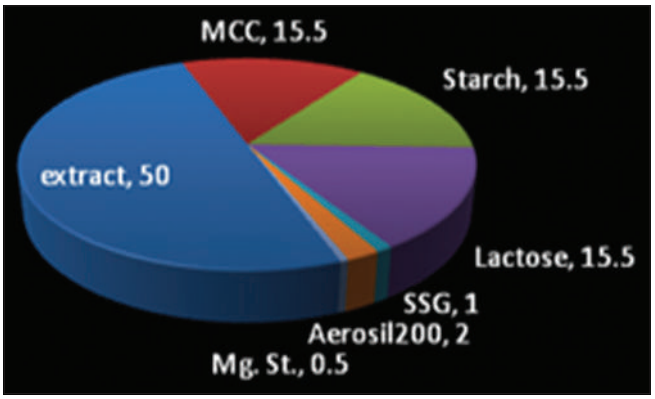


**Figure 2:** Ingredients of the final selected batch in percentage

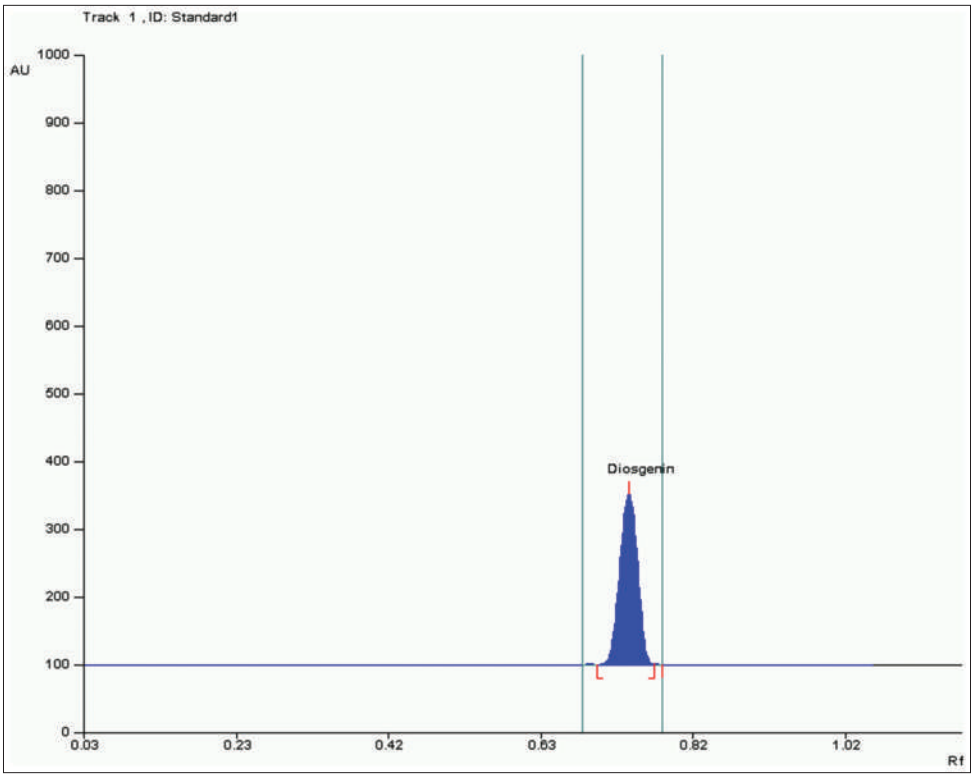


**Figure 3:** Densitogram of standard diosgenin at 194 nm

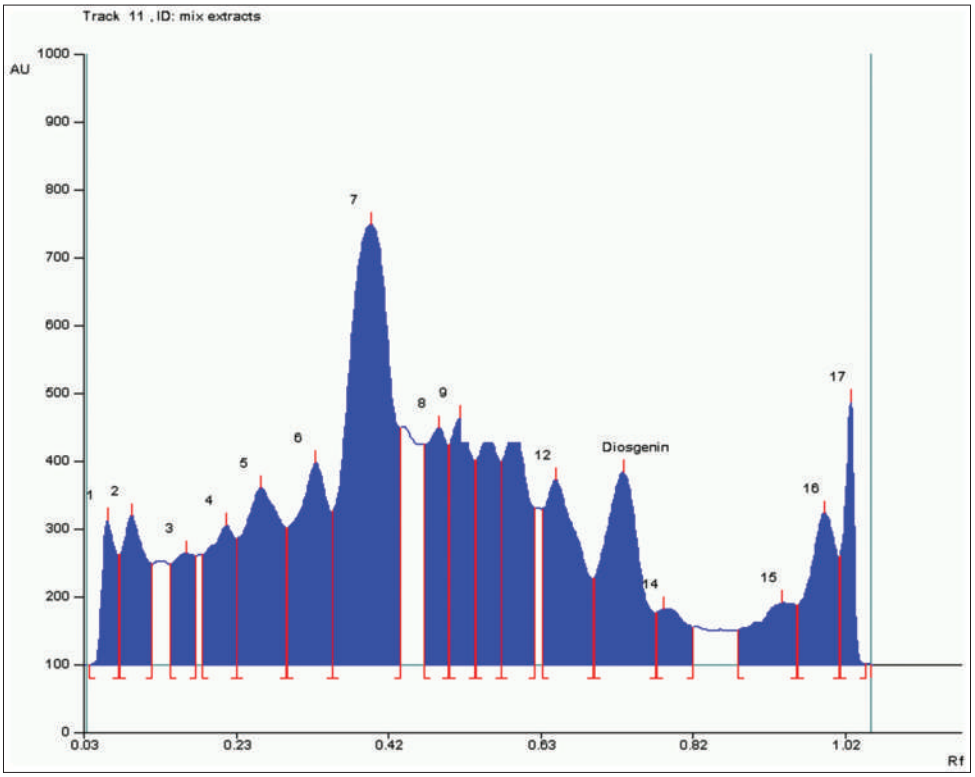


**Figure 4:** Densitogram of the mixture of extract at 194 nm

density of granules of the selected final batch were found to be 0.528 ± 0.04 and 0.644 ± 0.004, respectively. The mean values of compressibility index and Hausner's ratio of the final selected batch were found to be 18.013 ± 0.513 and 1.219 ± 0.007, respectively, angle of repose (θ) of final selected batch was found to be 27.136 ± 0.02.

### *Post-compression parameter*

The mean values of friability, hardness, disintegration of 15 batches prepared by wet granulation method are mentioned in Table 2, friability, hardness, and disintegration time of the final batch of extract tablet were found to be 0.279 ± 0.02, 11 ± 0.00 and 7.906 ± 0.16, respectively. Ingredient of final selected batch in is mentioned in Figure 2.

### *Uniformity of diameter*

The mean value of the diameter of the tablet was found to be 13 ± 00 mm.

### *Weight variation of extract tablet*

The mean value of randomly selected 20 tablets was found to be 799.45 ± 1.737 mg. The deviation of individual tablet weight from the average weight of 20 tablets was found within the percentage limit of 5%.

## Physicochemical Studies of Final Selected Batch of Extract Tablet

### *Organoleptic properties*

Appearance: Tablet (Slightly biconvex), color: Brownish white, smell little odorous, taste pungent, texture hard.

### *Ash value*

The mean percentage values of the total ash, acid insoluble ash, water-soluble ash, and sulfated ash of tablets of final selected batch were found to be 14.61 ± 0.137, 29 ± 0.02, 4.66 ± 0.08, and 14.37 ± 0.02, respectively.

### *Loss of weight on drying at 105°*

The mean percentage age value of loss of weight on drying at 105° of tablets of final selected batch was found to be 5.293 ± 0.14.

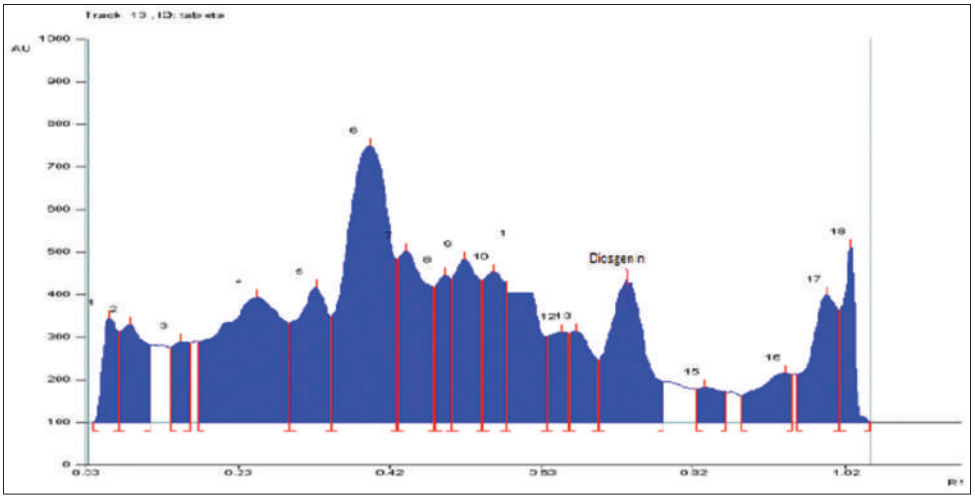


**Figure 5:** Densitogram of tablet at 194 nm

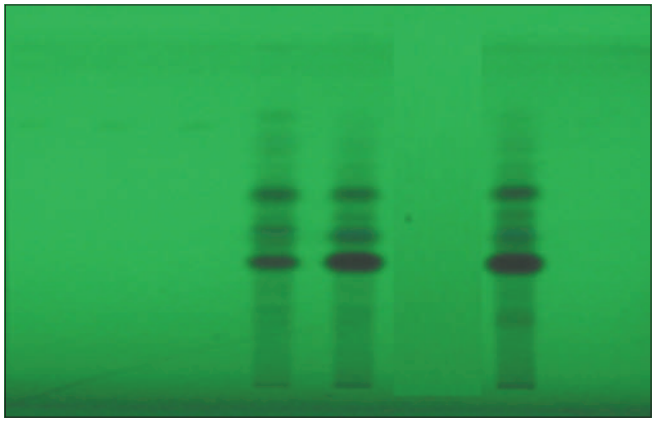

**Figure 6:** High-performance thin-layer chromatography pattern of extracts and tablet along with standard diosgenin at 254 nm (from R to L, Tab-mixed extract-*Tribulus terrestris*-Diosgenin)

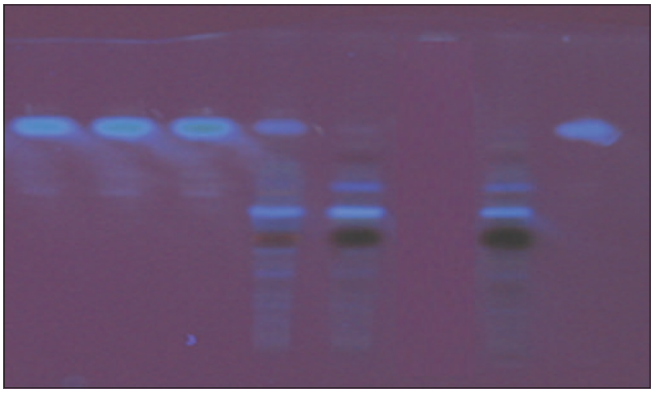

**Figure 7:** High-performance thin-layer chromatography pattern of extracts and tablet along with standard diosgenin at 366 nm after derivatization (after spraying with vanillin sulfuric acid) (from R to L, Tab-mixed extract-*Tribulus terrestris* Diosgenin)

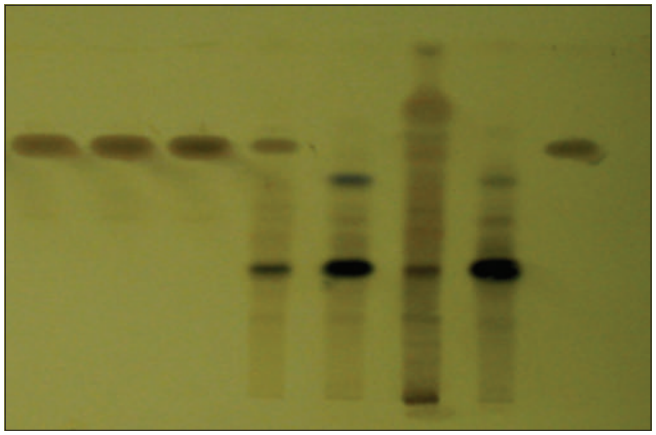

**Figure 8:** High-performance thin-layer chromatography pattern of extracts and tablet along with standard diosgenin at visible light after derivatization (after spraying with vanillin sulfuric acid) (from R to L, Tab-mixed extract-*Tribulus terrestris*-Diosgenin)

### *pH value*

The mean value of pH determined at 1% and 10% solution for extract tablets of the final selected batch was found to be 6.29 ± 0.00 and 5.56 ± 0.00, respectively.

### *Qualitative chemical test*

Test is done on SK indicated the presence of fixed oil and resinified volatile oils, flavonoids, glycosides, phenols, saponins, starch, steroids, tannins, resin, proteins, alkaloids, reducing sugars, carbohydrates, and terpenoids whereas resin was not detected.

### *HPTLC analysis*

HPTLC method was developed for standard diosgenin. The chromatogram of diosgenin showed linearity at 194 nm

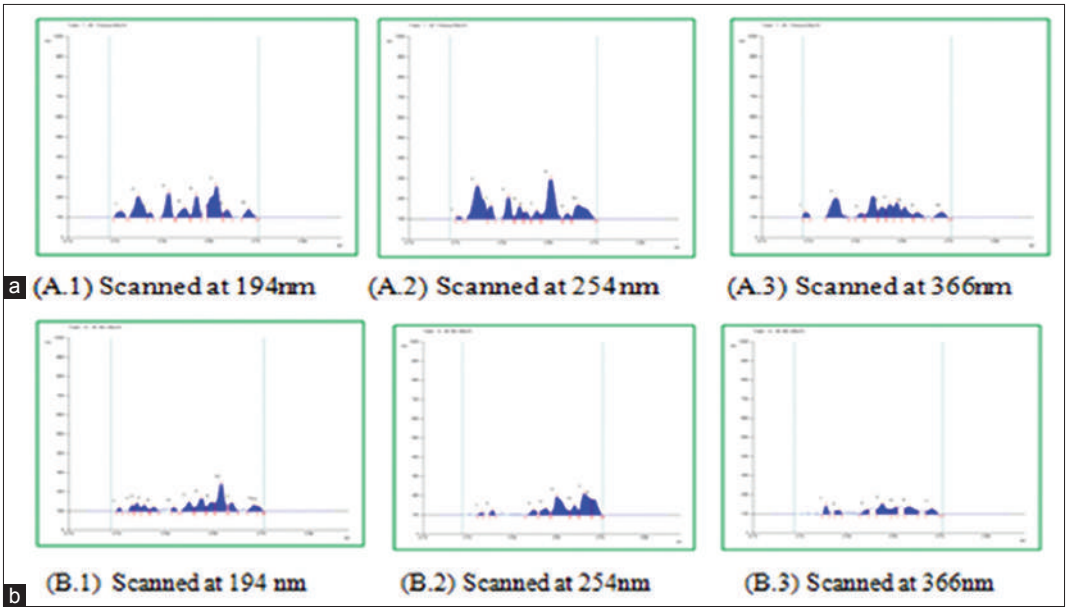


**Figure 9:** Chromatogram of the mixture of extract at 194 nm, 254 nm, and 366 nm, (a) dissolved in methanol (b) dissolved in chloroform

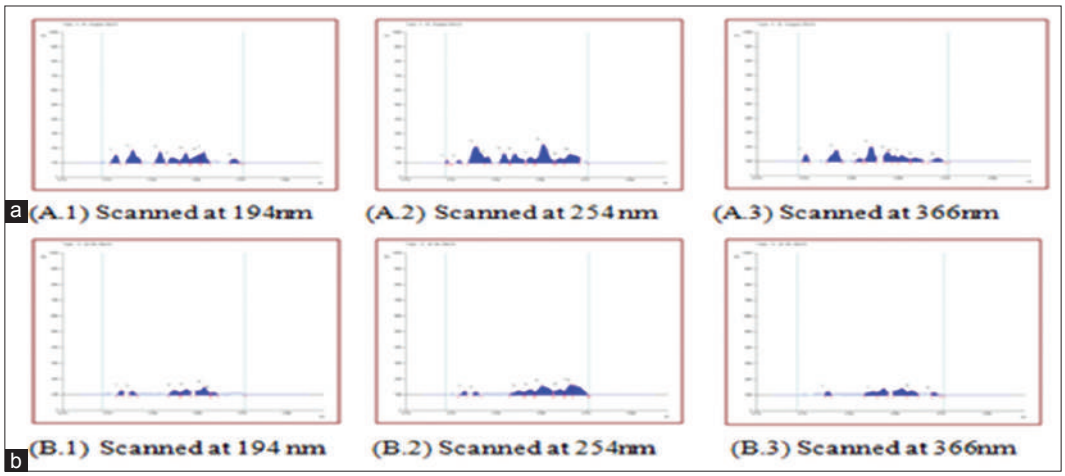


**Figure 10:** Chromatogram of tablet at 194 nm, 254 nm, and 366 nm, (a) dissolved in methanol, (b) dissolved in chloroform

(Calibration curve). Validated HPTLC method was applied to quantify the acid hydrolyzed extract of mixture of plant drugs (TT, *A. pyrethrum, and Z. officinale*) in the same proportion of tablet and tablet. Peaks for mixture and tablet are shown in Figures 3-5, and photos of HPTLC pattern are shown in Figures 6-8. Diosgenin contents estimated in plain TT were 144 µg/g, mixture of extracts 71.2 µg/g, and tablet 63.85 µg/g.

### *HPTLC fingerprinting of extracts / tablet*

Fingerprinting was done in methanol and chloroform to study the variation. HPTLC pattern is shown in Figures 9 and 10. The number of peaks at 194 nm, 254 nm, and 366 nm is shown in Table 3. At 194 nm, 254 nm, and 366 nm mixture of extract dissolved in methanol showing the same Rf value in tablet dissolved in methanol with well-defined correlation with their area percentage. Similarly, mixture of extract dissolved in chloroform and tablet dissolved in chloroform showed the compound with nearly same $R_f$ values with nearly same area percentage. Peak area percentage with same $R_f$ value in mixture of extracts (without excipients) and tablet are given in Figure 11.

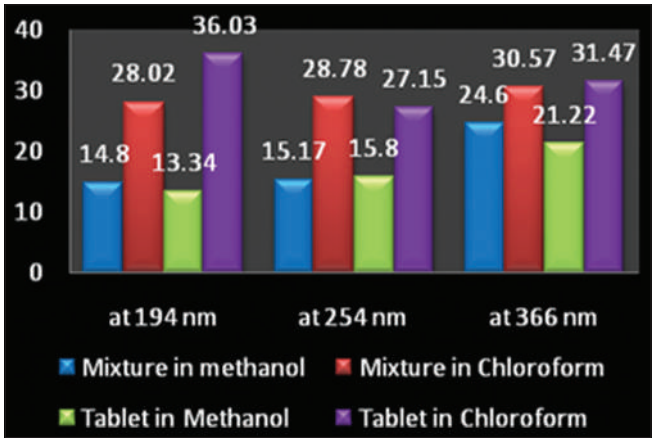


**Figure 11:** Area of constituents in percentage in the mixture at the same $R_f$ value in mixture and tablet

**Table 3:** Number of peaks of all samples at wavelength 194 nm, 254 nm, and 366 nm

| Sample | Dissolved in | 194 nm | 254 nm | 366 nm |
|---|---|---|---|---|
| *Tribulus terrestris* | Methanol | 8 | 10 | 8 |
| | Chloroform | 8 | 9 | 5 |
| *Zingiber officinale* | Methanol | 15 | 12 | 11 |
| | Chloroform | 12 | 10 | 11 |
| *Anacyclus pyrethrum* | Methanol | 10 | 11 | 8 |
| | Chloroform | 7 | 7 | 4 |
| Mixture | Methanol | 9 | 10 | 10 |
| | Chloroform | 13 | 8 | 7 |
| Tablet | Methanol | 8 | 10 | 9 |
| | Chloroform | 6 | 8 | 6 |

**Table 4:** Effect of SK (50:50 hydroalcoholic extract tablets) on mating behavior

| Parameters | Control Group I (1 ml dw.) | Treated Group II (SK 326.66 mg/kg bw.) | Treated Group III (extract tablet 140 mg/kg bw.) | Treated Group IV (extract tab 70 mg/kg bw.) # |
|---|---|---|---|---|
| Mounting frequency (MF) | 5.2±0.97 | 8±9.14 | 12.6±2.73* | 9.66±2.02 |
| Intromission frequency (IF) | 6.8±1.93 | 8±1.34 | 10±1.67 | 10±3.60 |
| Mounting latency (ML, in s) | 149±116.76 | 30.6±5.03 | 31±9.14 | 22±8.88 |
| Intromission latency (IL, in s) | 668.6±561.65 | 158.8±26.57 | 93.2±34.93 | 103.66±60.94 |
| Ejaculatory latency in first series (EL, in s) | 213.4±46.15 | 434±62.98 | 702.4±98.56** | 265±64.08 |
| Post ejaculatory interval (PEI, in s) | 438.8±129.10 | 480.8±81.03 | 258.8±53.76 | 147.66±1.33 |

*n*=5 (in each group), *$P<0.05$, **$P<0.01$ (Values are mean±standard error of the mean). SK: *Safoofe kharekhasak*

### *Effect on mating behavior test*

Data obtained with oral administration of SK and different doses of extract tablet are summarized in Table 4. Both classical drug (SK) Group II and treated (formulated tab) Group III showed significant increase in respect of mean observation in IL, MF, and IF with respect to control group, and ML and IL were found not significant. A significant effect was observed with a dose of 140 mg/kg BW, in treated Group III with increase in MF and ejaculatory latency. In contrast, with the higher dose in treated Group V at a dose of 280 mg/kg bw, all the behavioral parameters monitored were significantly altered there was impairment of sexual behavior, inhibition of locomotor activity and majority of male rats were sleepy, and they slept for maximum time of observation. In IV group mating was observed in three animals only so was not considered in statistical comparison analysis [Table 4].

## DISCUSSION

Modification of classical time tested dosage form can be debatable, and this debate also imply in the case of SK. Earlier Unani and Arab physicians also tried to experiment a lot in respect of dosage form, and there is a need to study classical dosage form with modification to assess the effect of conversion on its pharmacological activity and other aspects of formulations. These types of studies can reveal the added benefits of new conversions or can also validate the orthodox method. Qualitative chemical test done on SK indicates presence of flavonoids, saponins, steroids, alkaloids, etc. Several works suggest probable role of saponins (Androgenic/testosterone-like effects), alkaloids and sterols (nerve stimulating property), and flavonoids (increase blood flow to the testis) for aphrodisiac effect.[36]

Manufacturing of good quality extract tablets starts from the collection of good quality of raw material with efficient cleaning. The process of extraction when performed by Soxhlet apparatus, to obtain dry extract, liquid extract is further subjected to drying. The technique of drying depends on the stability of the active ingredients and the amount of moisture that must be removed.[37]

For drying rotary evaporator and for further drying hot air oven was used, further study can be conducted for the most appropriate method of drying for this combination. The dry extract was obtained for three of the drugs separately. In rotary evaporator, the centrifugal force and the frictional force between the wall of the rotating flask and the liquid sample results in the formation of a thin film of warm solvent being spread over a large surface. This rotation force suppresses bumping, it allows for quick, gentle evaporation of solvents in vacuum from most samples. Moreover, the solvent collected can also be reused.[38] The solvent used for extraction was 50:50 ethyl alcohol and water because various study showed aphrodisiac and androgenic activity in alcoholic and aqueous extracts of drugs present in the formulation[39-43] and among all the solvents 50% ethanol in water gave the highest extractable material weight.[37] The total dose of combined extract obtained was 1197.82 mg [Table 1] so an average 1200 mg of extract dose was incorporated in three-tab containing nearly 400 mg extract in each.

Initially, direct compression of the formulation powder was also attempted, but it does not comply with the pre- and post-compression parameters, then wet granulation technique was adopted. Wet granulation was observed better for this combination it is also widely practiced in manufacturing of tablet from botanical extract as it improves the flowability and compactness of high dose botanical drug substances it improves the processing characters of some botanical extracts which were observed in granulation batches which resulted in superior flow property compared to direct compression of extract powders.[37]

Different fillers (in different proportion), disintegrants, binders, lubricant desiccant, etc., are used in the formulation for making the granules, the pre-compression parameter of all the batches was evaluated, proper procedure granulation was adopted, granulation was done manually by hand with the help of sieve, granulator was not used due to more number of batches. Final selected batch displayed good pre-compression properties bulk density and tapped density, 0.528 and 0.644, respectively, Car's Index was 18.01 which was below 23 and is passable for good flow property, Hausner's ratio was 1.219 which is under good range for type of flow, angle of repose was 27.13 which is under good range.[18,20] One of the major challenges of powdered extract is poor flowability, proper flowability is very important in the processing of powders particularly when tablets are compressed in high-speed tablet presses, generally dry botanical extract shows poor flowability, in this study this issue was properly addressed by addition of proper lubricant, desiccant, glidant and processing which reflects in pre-compression parameters result. Hydrated silica (Aerosil-200) apart from other lubricant probably played an important role in this and also provided desiccant action in the tablets, generally, in botanical industry, it is a common practice to add silicon dioxide or maltodextrin to the soft extract.[37] Final batch of tablets selected on the basis of pre- and post-compression parameters contain drug and excipients in a ratio. Extract 400 mg (50%), MCC, starch and lactose 124 mg (15.5%) each SSG. 8 mg (1%), Aerosil-200 16 mg (2%), magnesium stearate 4 mg (0.5%) post-compression parameters were, Hardness in Kg, friability (%), disintegration time were 11.0 ± 0.00, 0.279 ± 0.02, and 7.906 ± 0.169, respectively, before this a batch with similar constituent was prepare with magnesium stearate (8 mg 1%) also showed good pre- and post-compression parameters but disintegration time tested was 9.55 min. After reducing magnesium stearate to half, the disintegration time was reduced and there was no effect on pre-compression parameters. magnesium stearate is hydrophobic; they work

by coating the component of the formulation and reduces the friction, but they can retard the penetration of water into the tablet and can extend the disintegration times and decrease the dissolution [Table 2].[44] Similarly, same batches are prepared with and without SSG [Table 2] (batch no. 13 and 15) batch with SSG displayed better disintegration time both reduction of magnesium stearate and inclusion of SSG results in fast disintegrating tablet, suiting to the pharmacological action expected from the formulated tab

Use of particular excipient in particular proportion apart from appropriate pre- and post-compression parameters may also be justified as in final batch quantity of lactose is less than other batch which contain higher quantity of lactose as keeping in mind lactose intolerance in some people, negative effect on phytochemical profile exhibited with lactose on storage which is lesser in MCC and degradation by microorganism as we have not used any preservatives in the formulation[37] [Table 2]. However, it is used in less quantity as it allows better mixing with other formulation ingredients and utilizes the binder more efficiently. Lactose monohydrate and corn starch can be used in tablets to improve compressibility, flowability, and disintegration properties also. MCC used in the final batch is used in pharmaceuticals as a binder/diluent in orally used in both wet-granulation and direct-compression processes, also has some lubricant and disintegrant properties that make it useful for tablet making it provides better compression character to the tablets. Starch used in the formulation is a multipurpose excipient; it was used primarily as a binder and was mixed after wetting; it also acts as diluent and disintegrant; it is one of the most commonly used tablet disintegrants. As filler/diluents, it is useful for, potent drugs, and herbal extracts, facilitating subsequent mixing or blending processes in manufacturing operations. It can also help improve powder flow (especially dried starches).[45,46] The study showed that using lactose or MCC in the formulations resulted in faster drug release profiles. This effect may be imparted through synergistic interactions between Starch and MCC and the filler actively forming an integral part within the MC gel structure. Formulations with lactose produced the highest ejection forces.[46,47]

The majority of tablet batch in wet granulation does not show major defect; no capping or lamination was observed in final batch [Table 2]. Final selected batch No. 15 was easily granulated and gave max yield with very minimum/few residues in sieve, shows good binding without any specific binder probably MCC and starch aided in for binding as per there activity, natural binding activity was also observed in the extracts. All the tablets in reported batches when kept in air tight container with silica pouch were dry and good on appearance.

It may be concluded that from mention ingredient/excipient and process, we can make tablets probably of high kinetic and dynamic quality addressing the analytical specification. Further stability study is needed with preservative and non-preservative variables as extract tablets are generally poor in stability, proper coating technique can be adopted with study on the coated version.

## HPTLC

Number of spots and $R_f$ value of each spot in a particular mobile phase are an index of purity and quality of drugs; it plays most important role to find out adulteration in drug. Hence, HPTLC study was done and a fingerprinting was set in for the tablet for future reference/work, including estimation of diosgenin which is a non-sugar component of saponin present in the formulation obtained after hydrolysis. Plane TT has diosgenin 144.5 µg/g. In tablet, diosgenin content was 63.6 µg/gm and in mixture of extract (ME), i.e., 71.2 µg/gm, slight decrease in diosgenin in tablet may be due to degradation in heating process or due to the excipient present in it. Diosgenin, which is a sapogenin from TT, was taken as a reference standard which is its essential component.[48]

HPTLC fingerprinting analysis at 194 nm, 254 nm, and 366 nm mixture of extract dissolved in methanol and chloroform showed the same $R_f$ value in tablet dissolved in methanol and chloroform with well-defined correlation with their area percentage, which indicates negligible variation in area percentage of major constituent after subjecting extracts to the chain of processes during tablet preparation, suggesting standard manufacturing process of extract tablet was adopted. These findings will further be helpful to set standard manufacturing process of extract tablets. Some peaks are missing in the tablet sample, for example, *Z. officinale* displays 15 peaks at 194 nm while tablet is displaying only eight peaks, which may be due to less quantity of ginger constituent in tablet after granulation process, or these constituents may have merged during the processing [Table 3].

## Effect of Formulation on Mating Behavior

Treated group tested for mating behavior showed marked changes in the male sexual behavior of rats. Very interesting observation was that the nature and severity of effects, differed according to the dose used; the lower dose treated group (Group IV) showed intromission only in three animal this may be due to other external factors; therefore, this group was not consider in statistical comparison analysis, only mean of data obtained from three animal is displayed in the table # [Table 4]. Whereas as in the higher dose in Group V in addition, decreased sexual performance, sexual interest and libido was observed and majority of animal were resting/sleeping with decrease in locomotor activity this effect may be due to sedative and related effect of formulation at this dose range, as some of the ingredient/drugs in the formulation like gingerols is reported for sedative effect[49] and also

myorelaxation activity of AP was observed compared to that produced by diazepam.[50] Thus, the ant masculine effects of treated Group V is not looking like a consequence of generalized toxic effects but is due to a selective action, as all the animals in each group were healthy throughout and after the study, increase in weight was observed in each group and no any mortality was noted during and after the study indicating safety of the formulation [Table 4]. Hormesis, in this case, not seems to be of toxicological origin, but is seems biphasic dose-response with stimulation in low and moderate dose with beneficial aphrodisiac effect and a high dose causing inhibitory or diverse effect as in this case sedative on observation.[51]

With the lower dose, ML was reduced, but IL was increased in comparison to Group III; this can be related to androgenic effect of TT in the formulation as androgenic effect of its aqueous extract was observed on chronic administration.[52] As the large intergroup variations were observed like all the behavioral studies, the non-significant effects in the test group seem inflated.

The tested formulation in the present study showed effect on sexual behavior by mating behavior. Significant sexual behavior enhancement was observed in formulated tablet in terms of MF and EL in comparison with control and classical formulation, prolongation of the ejaculatory latency by itself suggested an aphrodisiac action.[53]

This finding suggests a new indication of formulated tablets in premature ejaculation. There was increase in mean MF and IF in Groups II and III as compared to control. The MF and IF are considered as the indicator of libido and strength indicating that the test drug possesses an improving effect in sexual function. The test drug in treated group showed reduction in the ML and IL as compared to control; furthermore, this reduction was more in treated Group III further supporting sexual behavior enhancing effect of the formulated tablets. PEI is considered as an index of potency and libido and a parameter of the rate of recovery from exhaustion after first series of mating.[35]

Mean of PEI was found decreased more in treated Group III (formulated tablet). These all finding indicated that formulated tablets in converted dose from human are better than classical formulation in some observations such as IL, PEI, and MF, suggesting a better alternative to classical dosage form, particularly in the sugar restricted population. Libido, sexual vigor, and sexual performance were non normal during the aphrodisiac action and other activity, i.e., anogenital sniffing, grooming, and jumping was also observed during the work particularly in Groups II and III. Further mechanism of action of the formulation is needed to be established for enhancement of potency and libido related activity. Increase in fertilization was seen in Groups II and III after observation of animals post-experiment, as instance of pregnancy and magnitude of delivered rats were more in the two groups, but further detail fertilization study with sophisticated and elaborative parameters is needed in future to establish its fertility activity. Shortcoming comings of the work are stability and long-term safety/toxicity evaluation particularly long-term toxicity and complete profile evaluation of aphrodisiac, androgenic and pro-fertility activity with further sophisticated and accurate mechanism including further study on drug kinetics to further establish efficacy and utility of formulated tablet.

Decoction, majoon (Semisolid), pills, and Safoof (powder) are the common dosage form used in Unani medicine. Tablet or capsules made from powdered botanical raw material or extract are the current most popular form of botanical product/dosage form in the market.[37] This work is one such step for upliftment of the classical Unani formulation. Results of the animal study indicate that formulated tablets could be useful in the treatment of men with sexual dysfunctions resulting primarily from premature ejaculation and loss of Libido. Non sugar alternate of classical formulation can be of immense utility particularly in diabetics, interesting correlation of dehydroepiandrosterone sulfate level with the incidence of low sex drive and higher occurrence of impotence was discovered in studies of patients diagnosed with diabetes mellitus, and on review it was found that study on protodioscin from TT showed a significant increase of dehydroepiandrosterone sulfate levels in subjects with and without diabetes after treatment, and a significant increase in the frequency of successful intercourse of 60% in subjects with or without diabetes was noted.[54] Clinical trial with appropriate subjective and objective parameters is needed in future for its intensive utilization.

The dose comparing to classical formulation is reduced from 14 g unpleasant powder to three tablets nearly 800 mg each which is easy to swallow and will facilitate to comply the regimen. The finding the animal evaluation also suggests its use in erectile dysfunction. The chemical finding also suggests that functional group responsible for the activity was not degraded during the formulation procedure. Physicochemical data for formulated tablet were set in such as loss of weight on drying, pH, total ash, water-soluble, acid insoluble, and sulfated ash, extractive values, qualitative test for various functional groups, HPTLC fingerprinting and diosgenin estimation which can be used as standards for future reference.

## CONCLUSIONS

It can be concluded that formulated tablet from powder contains extract 400 mg (50%), MCC, starch and lactose 124 mg (15.5%) each SSG 8 mg (1%), Aerosil-200 16 mg (2%), magnesium stearate 4 mg (0.5%), it displayed most appropriate pre- and post-compression parameter with Car's Index 18.01, Hausner's ratio 1.219, aangle of repose 27.13. θ,

Hardness in Kg, friability (%), disintegration time were 11.0 ± 0.00, 0.279 ± 0.02, and 7.906 ± 0.169, respectively. The average weight of tablet was 799.45 ± 1.737 mg and weight variation was within the percentage limit, i.e., 5%. Values for various physicochemical parameters were set in for formulated tablets with quantitative estimation of diosgenin and qualitative HPTLC fingerprinting for further quality control work. Mating behavior study reveals significant effect of the formulated tablet with the dose (140 mg/kg BW) in MF and ejaculatory latency. Effect on mating behavior in rats indicates that formulated tablets could be useful in the treatment of sexual dysfunctions resulting primarily from premature ejaculation and loss of libido in place of Safoof powder form alternately. Finding of both formulated tablet and powder validates the claim of aphrodisiac activity mention in Unani medicine.

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