

Physicochemical Standardization of Polyherbal Powder Formulation: *Safoof-e-Makhana*

Gazi Jahangeer Rather, Hamiduddin*, Mohd Ikram, Shaista Fatima, MD Naquibuddin

ABSTRACT

Introduction: *Safoof-e-Makhana* (SM) is a Unani polyherbal powder formulation used to treat Sexual disorders. Formulation consists of following herbs *Orchis latifolia*, *Hygrophila auriculata*, *Asparagus racemosus* and *Eleitaria cardamomum*. The main aim of this study was to standardize *Safoof-e-Makhana* (SM), on the basis of organoleptic characters and physico-phytochemical analysis. **Methods:** The drugs were cleaned, dried in shade and powdered by passing through sieve # no. 80 as per the method described in UPI / National Formulary of Unani Medicine. This *Safoof* formulation was evaluated using physicochemical tests: powder characterization, extractive value, alcohol and water soluble matter, Ash value, LOD at 105°C, pH and HPTLC fingerprinting. Statistical analysis used: Mean \pm SEM. **Results:** Organoleptic characters of the formulation are light brown colour, characteristic odour, sweet taste and moderately fine texture. Physicochemical parameters displayed water soluble extractive (21.83 \pm 0.08), alcohol soluble extractive (7.87 \pm 0.09), total ash (4.76 \pm 0.08), acid insoluble ash (3.36 \pm 0.01), water soluble ash (0.89 \pm 0.25), LOD at 105°C (11.38 \pm 0.34), pH of 1% and 10% solution were 6.6 \pm 0.1 and 6.0 \pm 0.1 respectively. Phytochemical qualitative analysis displayed presence of alkaloids, tannins, flavanoids, steroids, terpenoids, carbohydrates, volatile oil. HPTLC fingerprinting data was also set in. **Conclusion:** The standardization of this formulation was done and the data obtained would be used as a standard for future reference.

Key words: Physicochemical, Polyherbal, Powder, Formulation, Standardization, *Safoof-e-Makhana*, Unani Medicine.

Key messages: Standardization of herbal drugs is very important for quality control. It improves the safety and efficacy of drug to provide the best possible medicine to the society. Obtained data for *Safoof-e-Makhana* will be useful as standards for quality control of formulation.

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INTRODUCTION

Standardization of herbal formulations is essential in order to assess quality, purity and efficacy of drugs. The quality assessment of herbal formulations is of great importance in order to justify their acceptability in contemporary period of medicine.¹ One of the major problems faced by the herbal industry is the unavailability of strict quality control measures for herbal medicines and their formulations. The World Health Organization (WHO) has appreciated the role and importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member countries in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.² There is a tremendous role and scope in Indian traditional medicine including Unani system of medicine (USM) for production of standardized, therapeutically effective formulations. There is a need to explore the medicinally important plants and their formulations. This can be achieved only if the herbal products are analysed and evaluated using sophisticated modern techniques of standardization.³ Quality evaluation of herbal preparation is a

fundamental requirement of pharmaceutical industry and other organization dealing with Unani and herbal products.

Safoof-e-Makhana (SM) is a polyherbal powder formulation used in the Unani System of Medicine for treatment of sexual disorders since ancient time and the formulation is mentioned in National Formulary of Unani Medicine.⁴ This formulation is not evaluated for its physicochemical and phytochemical standardization since yet as revealed by the literature survey. Thus keeping this goal in mind, the present study was carried out to fix the quality control standards of *Safoof-e-Makhana* (SM) with scientific analytical techniques.

MATERIALS AND METHODS

Procurement of raw drugs

Ingredients of *Safoof-e-Makhana* (SM) were procured from the pharmacy of NIUM and authentic shop of Bangalore, Karnataka, India. The identification of these drugs was done by the experts at National Institute of Unani Medicine Kottigepalya, Bangalore.

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The ingredients of *Safoof-e-Makhana* (SM) viz. *Orchis latifolia* Linn. (SalabMisri), *Hygrophila auriculata* (Schum.) Haine (Tukhm-e-Talmakhana), *Asparagus racemosus* Willd. (Satawar) and *Elettaria cardamomum* Maton. (Ilaichi Khurd) are present in equal proportion.

Preparation of Formulation

All the drugs were first cleaned and dried in shade and powdered by passing through sieve # no. 80. The formulation [Figure 1] was prepared as per the method described in UPI / National Formulary of Unani Medicine.⁴

Organoleptic evaluation

The Organoleptic evaluation refers to evaluation of the *Safoof-e-Makhana* (SM) formulation by colour, odour, taste, appearance, particle size and texture.⁵

Powder characterization⁶⁻⁷

Angle of Repose: The angle of repose gives an indication of the flow ability of the substance. Funnel was adjusted such that the stem of the funnel lies 2 cm above the horizontal surface. The drug powder was allowed to flow from the funnel under the gravitational force till the apex of the pile just touched the stem of the funnel, so the height of the pile was taken as 2 cm. Drawing boundary along the circumference of the pile and taking the average of six diameters determined the diameter of the pile. These values of height and diameter were then substituted in the following equation:

$$\text{Angle of Repose } (\theta) = \tan^{-1} [2h/d]$$

Where, h - Height of the pile and d - Diameter of the pile

The experiment was done in triplicate.

Bulk Density and Tapped Density: The weighed quantity (20 gm) of *Safoof-e-Makhana* (SM) is carefully put into a measuring cylinder without any losses. The initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume gives the value of tapped density.

Carr's Index: Carr's index has been used as an indirect method of quantifying powder flow ability from bulk density; this method was developed by Carr. The percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and stability, and is calculated according to following equation. Carr's index (% compressibility) = $100 \times (1 - D_t / D_b)$ Where D_b = Bulk density, D_t = Tapped density

Hausner's Ratio: Hausner ratio has been also used as indirect method of quantifying powder flowability from bulk density. Hausner ratio = D_t / D_b . Where D_b = Bulk density and D_t = Tapped density.

Physico-chemical evaluation

The Physico-chemical evaluation of prepared *Safoof-e-Makhana* (SM) was done by testing loss of weight on drying at 105°C, total ash, acid insoluble ash, water soluble ash, pH of 1% and pH of 10% solution and extractive values.

Loss on drying at 105°C

An accurately weighed 3g of *Safoof-e-Makhana* (SM) was taken in a petri dish. The crude drug was heated at 105°C in an oven till a constant weight and percentage moisture content of the sample was calculated with reference to the weighed *Safoof-e-Makhana* (SM) sample.⁸

Ash Values, Determination of total ash acid, insoluble ash, water soluble ash is done as per protocol for testing of ASU drug and UPI^{5,8}

Determination of pH

1% and 10% solution of *Safoof-e-Makhana* (SM) was prepared in distilled water (w/v) and pH was determined by using digital pH meter.⁹

Extractive values⁸

Water soluble extractives: Five grams of *Safoof-e-Makhana* (SM) was macerated with 100 ml of water in closed conical flask for 24 h, shaken frequently for the first 6 h and allowed to stand for 18 h. This was filtered through filter paper. Twenty-five millilitres of the filtrate was evaporated to dryness in petri dish, dried at 105°C, and weighed. Percentage of water soluble extractive with reference to air-dried material was calculated.

Alcohol soluble extractives: Five grams of *Safoof-e-Makhana* (SM) was macerated with 100 ml of 70% ethanol in a closed conical flask for 24 h, shaken frequently during the first 6 h, and allowed to stand for 18 h. This was filtered rapidly taking precaution against loss of ethanol. Twenty five millilitres of the filtrate was evaporated to dryness in a petri dish, dried at 105°C, and weighed. Percentage of alcohol soluble extractive was calculated with reference to air-dried drug.

Successive extractive value and Non-Successive extractive value¹⁰

Successive extractive value: The coarse powder of *Safoof-e-Makhana* (SM) was extracted successively using soxhlet apparatus with different solvent, in increasing order of polarity, petroleum ether → benzene → chloroform → ethanol. 10 g powdered drug was taken and subjected to successive extraction with each solvent for 6 h. After that the extracts were filtered first by using filter paper (Whatman No. 1) and dried on water bath. The extractive values were determined with reference to the weight of the drug taken (w/w). The procedure was repeated 3 times to calculate mean extractive values.

Non successive extractive value: The coarse powder of *Safoof-e-Makhana* (SM) was extracted separately in different solvent (water, ethyl alcohol and petroleum ether) using soxhlet apparatus. 10 g powdered drug was taken and subjected to separate extraction with each solvent. The extracts were filtered first by using filter paper (Whatman No. 1) and

Table 1: Powder characterization of *Safoof-e-Makhana*.

S.no.	Parameters	Percentage mean (n=3) ± SD
1.	Bulk Density (gm/ml)	0.4442 ± 0.0001
2.	Tapped Density (gm/ml)	0.4876 ± 0.0001
3.	Carr's index	8.8876 ± 0.0008
4.	Hausner's Ratio	1.0974 ± 0.0001
5.	Angle of Repose	36.49 ⁹ ± 0.22

Table 2: Physicochemical characteristics of *Safoof-e-Makhana*.

S.no.	Parameters	Percentage mean (n=3) ± SD
1.	Loss on drying (%)	11.38 ± 0.34
2.	Ash Content	
A	Total Ash (% w/w)	4.76 ± 0.08
B	Water soluble Ash (% w/w)	0.89 ± 0.25
C	Acid Insoluble Ash (% w/w)	3.36 ± 0.01
3.	pH	
A	pH (1%)	6.6 ± 0.1
B	pH (10%)	6.0 ± 0.1

Table 3: Successive Extraction and Non-Successive Extraction.

Mean± SEM	Successive extractive value (%w/w)				Non-Successive extractive value(%w/w)				
	Petroleum ether	Benzene	Chloroform	Ethanol	Petroleum ether	Benzene	Chloroform	Ethanol	Water
	11.47±0.17	0.57±0.09	0.54±0.05	2.27±0.09	11.69±0.11	9.02±0.05	9.55±0.04	12.90±0.05	32.08±0.06

Table 4: Phytochemical Screening (Qualitative Estimation) of *Safoof-e-Makhana*.

Parameters	Result
Alkaloids	+
Glycosides	+
Tannins	+
Steroids	+
Flavanoids	+
Sugars	+
Phenols	+
Proteins	+
Saponins	+
Resins	+
Terpenoids	+
Anthraquinones	+
Essential oils & Fatty acids	+

evaporate on water bath. Extractive values were determined with reference to drug taken (w/w).

Qualitative Estimation of constituents

Qualitative estimation *Safoof-e-Makhana* (SM) for organic constituent's viz. alkaloids, glycosides, tannins, flavonoids, sugars, saponins, phenols, proteins, resins and steroids was done.⁹

HPTLC fingerprinting analysis

The weighed quantity (5 g) of *Safoof-e-Makhana* (SM) was extracted in a Soxhlet apparatus for 6 h using 100 ml of solvent (ethanol) at a controlled temperature. HPTLC was performed on 20 cm × 10 cm aluminium backed plates coated with silica gel 60F254 (Merck, Mumbai, India). Standard solution of sample solution were applied to the plates as bands by use of a Camag (Muttentz, Switzerland) Linomat V sample applicator equipped with a 100 µl Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28 ± 2°C in two different mobile phases separately viz. n-Butanol: Acetic acid: water (5:1:4) and Toluene: Ethyl acetate: Formic acid (5:4:1) (v/v), in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were dried and then scanned at 254 nm and 366 nm with a Camag TLC Scanner with WINCAT software, using the deuterium lamp.¹¹

RESULTS

The organoleptic properties of *Safoof-e-Makhana* showed the colour was light brown, odour was characteristic, taste was sweet, texture was fine and particle size was 80#. The powder characterization of *Safoof-e-Makhana* is depicted in [Table 1]. The physicochemical Evaluation of *Safoof-e-Makhana* is mentioned in [Table 2] and water and alcohol soluble extractive values were found to be **21.83 ± 0.08** (%w/w) and **7.87 ± 0.09** (%w/w) respectively. Successive and Non-Successive extraction,

Phytochemical Screening and HPTLC of *Safoof-e-Makhana* (SM) is depicted in Table 3, 4 and 5, 6, 7, 8 respectively.

DISCUSSION

Herbal formulations are considered harmless traditionally and being consumed increasingly by people without prescription. However, some can cause health problems, some are ineffective and some may interact with other drugs. Standardization of herbal formulations is essential in order to assess the quality and purity of drugs, based on the concentration of their active principles.¹³

Standardization is an important measure for knowing the quality, purity and for sample identification. It is essential for the correct identity of the materials.

Standards for *Safoof-e-Makhana* (SM) was generated in this work. Finished product of *Safoof-e-Makhana* (SM) was light brown in colour as per colour chart (No. PMS 141 of Pantone color chart).¹⁴ sweet in taste, characteristic in odor and without any clumping and aggregation.

The mean values of bulk density, tapped density, angle of repose, Hausner's ratio and compressibility index were 0.4442 ± 0.0001 , 0.4876 ± 0.0001 , $36.49^\circ \pm 0.22$, 1.0974 ± 0.0001 and 8.8876 ± 0.0008 respectively. Powder characterization methods i.e. Hausner's ratio and compressibility index are the simple and popular method to determine the flow characteristics of powder. The flow characteristics of powder depend on the size, shape and size distribution of particles.¹⁵ The compressibility index of *Safoof-e-Makhana* (SM) lies below 10 according to the scale of flowability, it shows the test drug SM has a excellent flow character. The Hausner's ratio of *Safoof-e-Makhana* (SM) lies in between 1.00 – 1.11, it indicates excellent flowability.¹⁶⁻¹⁷ Angle of repose displayed Passable flow property.¹⁷ [Table 1].

The mean percentage of loss of weight on drying of *Safoof-e-Makhana* (SM) is 11.38 ± 0.34 . [Table 2] It is mentioned that the water content in plant drugs can vary between 8% and 14%. The presence of excessive amount of moisture in plant drugs causes hydrolysis of constituents, growth of bacteria and fungi and biochemical reactions. The pharmacopoeial monographs compulsorily limit for the water content, especially in drugs that have hygroscopic nature, or in which the excessive amounts of water causes deterioration of products.¹⁸ The formulation having less amount of moisture can be expected to be safe for longer time.

The ash value is an important parameter in the quality control of herbal drugs. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. The total ash of SM was found to be 4.76 ± 0.08 % w/w. is reasonably low indicating low contamination. Water-soluble ash is the part of the total ash content, which is soluble in water. It is a good indicator of either previous extraction of water-soluble salts in the drug or incorrect preparation. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water. The acid insoluble and water-soluble ash values of SM were **3.36 ± 0.01** % w/w and **0.89 ± 0.25** % w/w respectively.¹⁹ [Table 2] pH of SM in 1% solution was **6.6 ± 0.1** while the pH of 10% solution was **6.0 ± 0.1**. [Table 2] It is slightly acidic in nature. The correlation between the pH and microbial contamination was studied by Abba et al. and suggested that a neutral or alkaline pH favours high microbial contamination levels of the herbal preparations.²⁰

Table 5: Rf value, No. of Peaks, peak area and height of ethanolic extract of *Safoof-e-Makhana* (SM) in n-Butanol: Acetic acid: water at 254nm.

Peak	Start Rf	Start Height	Max. Rf	Max. Height	Max. %	End Rf	End Height	Area	Area %
1.	0.24	0.0	0.25	12.2	2.01	0.28	1.7	185.5	0.76
2.	0.35	0.1	0.40	106.3	17.44	0.44	21.8	3351.1	13.72
3.	0.44	22.2	0.56	491.0	80.56	0.60	0.4	20888.3	85.52

Table 6: Rf value, No. of Peaks, peak area and height of ethanolic extract of *Safoof-e-Makhana* (SM) in n-Butanol: Acetic acid: water at 366 nm.

Peak	Start Rf	Start Height	Max. Rf	Max. Height	Max. %	End Rf	End Height	Area	Area %
1.	0.08	2.6	0.11	78.0	8.40	0.13	22.9	1126.1	2.76
2.	0.14	22.9	0.14	24.7	2.66	0.18	6.9	511.1	1.25
3.	0.18	7.1	0.20	21.7	2.34	0.23	8.5	537.7	1.32
4.	0.32	8.3	0.33	12.5	1.35	0.34	1.0	118.6	0.29
5.	0.34	0.1	0.40	145.8	15.70	0.44	65.4	5536.5	13.59
6.	0.44	65.5	0.56	645.9	69.56	0.60	0.7	32910.4	80.78

Table 7: Rf value, No. of Peaks, peak area and height of ethanolic extract of *Safoof-e-Makhana* (SM) in Toluene: Ethyl acetate: Formic acid at 254 nm.

Peak	Start Rf	Start Height	Max. Rf	Max. Height	Max. %	End Rf	End Height	Area	Area %
1.	0.49	2.3	0.52	14.8	6.99	0.54	10.2	319.5	5.51
2.	0.57	18.4	0.60	41.9	19.75	0.60	40.0	679.6	11.72
3.	0.60	40.3	0.63	116.6	54.91	0.68	43.8	3553.8	61.30
4.	0.73	32.1	0.75	39.0	18.36	0.79	0.8	1244.2	21.46

Table 8: Rf value, No. of Peaks, peak area and height of ethanolic extract of *Safoof-e-Makhana* (SM) in Toluene: Ethyl acetate: Formic acid at 366nm.

Peak	Start Rf	Start Height	Max. Rf	Max. Height	Max. %	End Rf	End Height	Area	Area %
1.	0.49	0.6	0.52	27.9	11.47	0.54	10.9	506.6	8.47
2.	0.56	15.3	0.60	38.6	15.90	0.61	35.7	926.7	15.50
3.	0.61	35.8	0.63	129.7	53.44	0.67	43.1	3488.6	58.34
4.	0.67	43.3	0.68	46.6	19.19	0.73	5.4	1057.7	17.69

The mean percentage of water-soluble and alcohol soluble extractive values of *Safoof-e-Makhana* (SM) were 21.83 ± 0.08 and 7.87 ± 0.09 respectively. The mean percentage of successive extractive values in petroleum ether, benzene, chloroform and ethyl alcohol were 11.47 ± 0.17 , 0.57 ± 0.09 , 0.54 ± 0.05 and 2.27 ± 0.09 respectively. Non-successive extractive values in petroleum ether, benzene, chloroform, ethyl alcohol and water were 11.69 ± 0.11 , 9.02 ± 0.05 , 9.55 ± 0.04 , 12.90 ± 0.05 and 32.08 ± 0.06 respectively. [Table 3] Extractive value of a drug in definite solvent is an index for checking the purity of a drug. Amount of the extract of a drug in a particular solvent is often an appropriate measuring tool for certain constituent in the drug.²¹

Organic constituent's viz. alkaloids, glycosides, tannins, flavanoids, sugars, saponins, phenols, proteins, resins, steroids were found present on qualitative estimation tests. [Table 4]

HPTLC: HPTLC plates of ethanolic extract of *Safoof-e-Makhana* (SM) in two separate mobile phases viz. n-Butanol: Acetic acid: water (5:1:4) and Toluene: Ethyl acetate: Formic acid (5:4:1) were examined. Rf value, numbers of peaks, peak area and peak height of *Safoof-e-Makhana* (SM) in two separate mobile phases viz. n-Butanol: Acetic acid: water (5:1:4) Figure 2, 3, 4, 5, 6 and Toluene: Ethyl acetate: Formic acid (5:4:1) Figure 7, 8, 9, 10 and 11 were also analysed under 254nm, 366nm respectively. Area percentage of peak no. 3 of *Safoof-e-Makhana* (SM) analysed under 254nm in n-Butanol: Acetic acid: water (5:1:4) was highest (85.52%).

[Table 5] Area percentage of peak no. 3 of *Safoof-e-Makhana* (SM) analysed under 254nm in Toluene: Ethyl acetate: Formic acid (5:4:1) was highest (61.30%). [Table 7] Area percentage of peak no. 6 of *Safoof-e-Makhana* (SM) analysed under 366nm in n-Butanol: Acetic acid: water (5:1:4) was highest (80.78%). [Table 6] Area percentage of peak no. 3 of *Safoof-e-Makhana* (SM) analysed under 366nm in Toluene: Ethyl acetate: Formic acid (5:4:1) was highest (58.34%). [Table 8]

Further studies can be done by the help of standards for quantitative estimation and identification of the ingredients peak. Present HPTLC fingerprinting data can help in authentication and identification of *Safoof-e-Makhana* (SM) in the performed solvent system and extract.

Review of literature on ingredient of SM revealed promising constituent and pharmacological activity in it. Safoof ingredient such as *Orchis latifolia* contains many important constituents like glycosides, starch, albumin, volatile oil and bitter principles; *Hygrophila auriculata* contains alkaloids and fatty acids;²² *Asparagus racemosus* contains Sugar, Glycosides, Saponins and Sitosterol.²³ *Elettaria cardamomum* contains Essential Oil.²⁴ Reported activity of aqueous extract of *Orchis latifolia* are ameliorative and aphrodisiac activities tested *in vivo*,²⁵ methanolic extract of *Orchis latifolia* shows anticandidal and antimicrobial activities tested *in vitro*.²⁶ Reported activity of aqueous and methanolic extract of *Hygrophila auriculata* shows antimicrobial activity tested *in vitro*,²⁷ alcoholic extract of *Hygrophila auriculata* shows antioxidant activity



Figure 1: *Safoof-e-Makhana*.

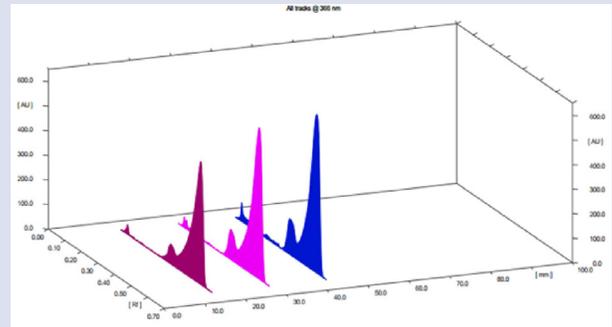


Figure 4: HPTLC 3-D Densitometric Scan of ethanolic extract of *Safoof-e-Makhana* (SM) in n- Butanol: Acetic acid: water at 366 nm.

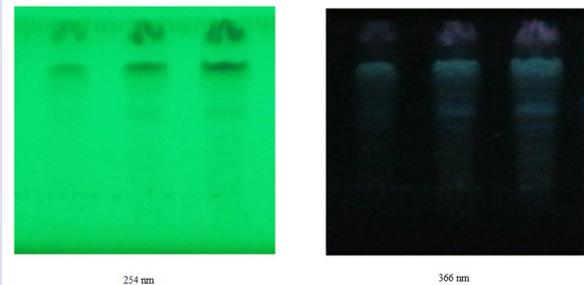


Figure 2: HPTLC photos of *Safoof-e-Makhana* (SM) ethanolic extract in n-Butanol: Acetic acid: water at 254 nm and 366 nm.

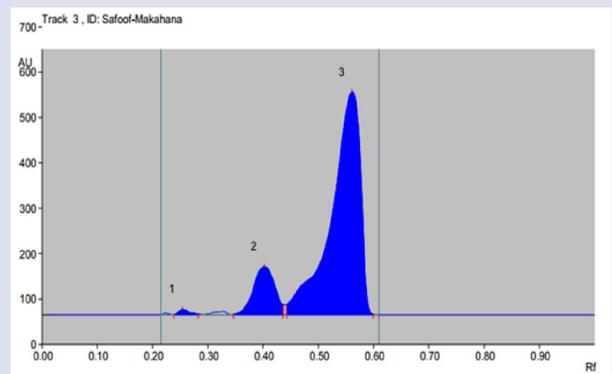


Figure 5: HPTLC fingerprint profile of ethanolic extract of *Safoof-e-Makhana* (SM) in n-Butanol: Acetic acid: water at 254 nm.

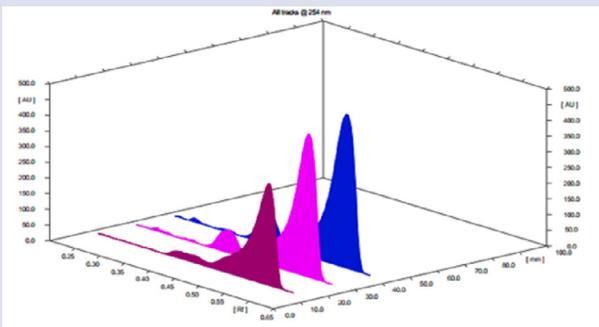


Figure 3: HPTLC 3-D Densitometric Scan of ethanolic extract of *Safoof-e-Makhana* (SM) in n-Butanol: Acetic acid: water at 254 nm.

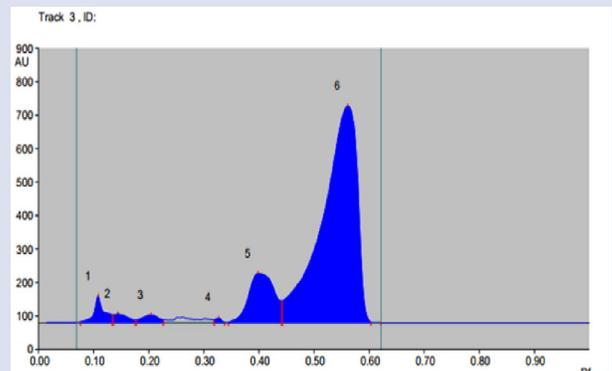


Figure 6: HPTLC fingerprint profile of ethanolic extract of *Safoof-e-Makhana* (SM) in n- Butanol: Acetic acid: water at 366 nm.

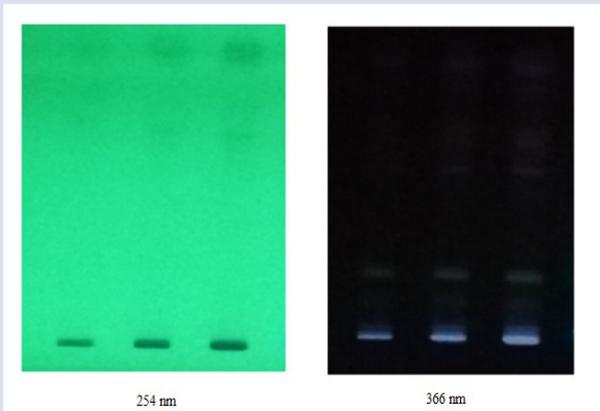


Figure 7: HPTLC photos of *Safoof-e-Makhana* (SM) ethanolic extract in Toluene: Ethyl acetate: Formic acid at 254 nm and 366 nm.

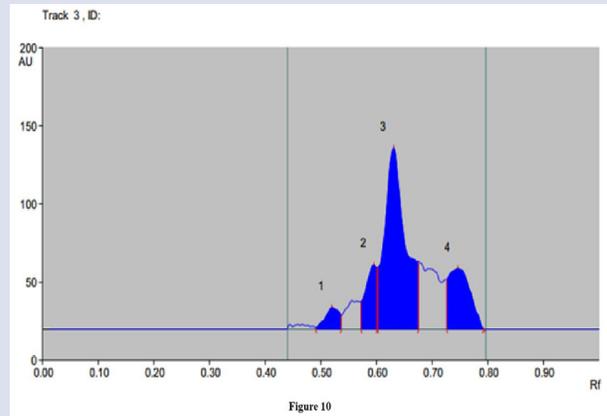


Figure 10: HPTLC fingerprint profile of ethanolic extract of *Safoof-e-Makhana* (SM) in Toluene: Ethyl acetate: Formic acid at 254 nm.

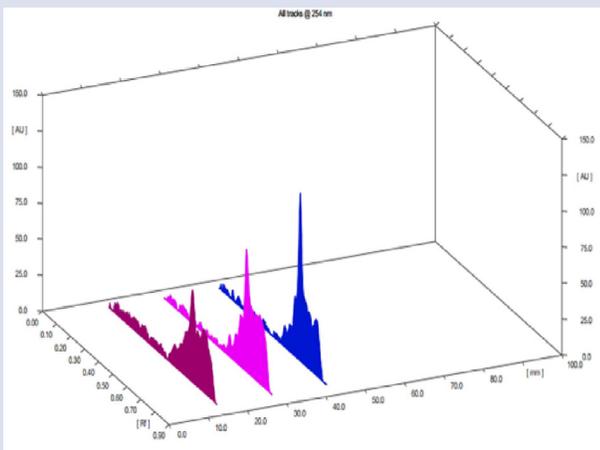


Figure 8: HPTLC 3-D Densitometric Scan of ethanolic extract of *Safoof-e-Makhana* (SM) in Toluene: Ethyl acetate: Formic acid at 254 nm.

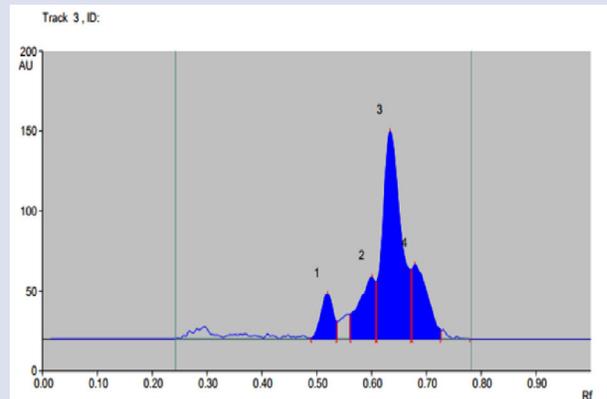


Figure 11: HPTLC fingerprint profile of ethanolic extract of *Safoof-e-Makhana* (SM) in Toluene: Ethyl acetate: Formic acid at 366 nm.

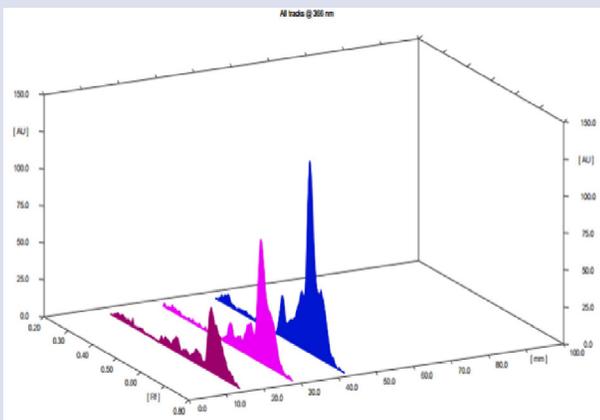


Figure 9: HPTLC 3-D Densitometric Scan of ethanolic extract of *Safoof-e-Makhana* (SM) in Toluene: Ethyl acetate: Formic acid at 366 nm.

tested *in vitro*.²⁸ Reported activity of hydroalcoholic extract of *Asparagus racemosus* shows aphrodisiac activity tested *in vivo*,²⁹ methanolic extract of *Asparagus racemosus* shows anti-oxidant and anti-ulcer activity tested *in vivo*.³⁰ Reported activity of aqueous extract of *Elettaria cardamomum* shows antimicrobial activity tested *in vitro*,³¹ methanolic extract of shows anti-oxidant activity tested *in vitro*.³²

The most important lacunae in alternative system of medicine for its globalization is difficulty to ensure uniformity and quality of drugs. Powder dosage form *Safoof-e-Makhana* (SM) doesn't have any pharmacopoeial standards since yet. Various methods and parameters for the assessment of powder dosage form are mentioned in different guidelines. It is necessary to follow these guidelines so that data can be generated and could be used to set the standards of the formulation. It can be used for quality control purpose to achieve maximum efficacy and safety of medicine.

CONCLUSION

In present study *Safoof-e-Makhana* (SM) was evaluated physico-chemically to set its physicochemical standards (loss of weight on drying, pH, total ash, water soluble, acid insoluble ash, extractive values, qualitative data for presence of organic constituents and HPTLC finger printing), which may be used as standard monograph for identification and quality

control and also further evaluation or future research work on standardization of this formulation.

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CONFLICT OF INTEREST

There are no conflicts of interest.

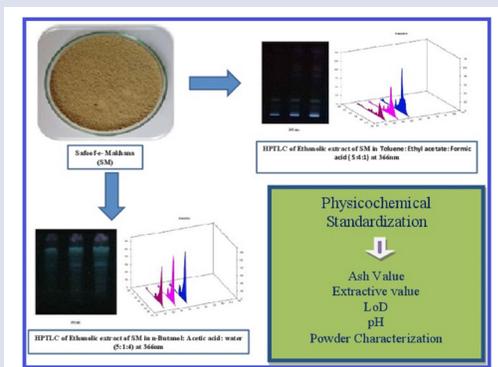
ABBREVIATIONS

WHO: World Health Organisation, **USM:** Unani System of Medicine, **SM:** *Safoof-e-Makhana*, **HPTLC:** High Performance Thin Layer Chromatography, **SEM:** Standard Error of Mean; **LOD:** Loss on Drying; **UPI:** Unani Pharmacopoeia of India; **ASU:** Ayurveda, Siddha and Unani; **TLC:** Thin Layer Chromatography.

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GRAPHICAL ABSTRACT



SUMMARY

- Standardization data of *Safoof-e-Makhana* (SM), a Unani pharmacopoeial Powder formulation composed of *Orchis latifolia*, *Hygrophila auriculata*, *Asparagus racemosus* and *Elettaria cardamomum* by various physicochemical parameters was established
- HPTLC analysis was performed and HPTLC figure printing data of ethanolic extract of SM in n-Butanol: Acetic acid: water (5:1:4) and Toluene: Ethyl acetate: Formic acid (5:4:1) were set in.

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