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STANDARDIZATION WITH HPTLC ANALYSIS OF POLYHERBAL POWDER FORMULATION: *SAFOOF-E-MUSAKKIN*

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Keywords:

Standardization, *Safoof-e-musakkin*, Physico-chemical

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ABSTRACT: Background & Objectives: Standardization of polyherbal Unani formulations is essential to assess the quality of drugs, based on their active principles, physical and chemical standards. This work reports on standardization of a polyherbal Unani powder formulation Safoofe- Musakkin (SM) consist of *Rauwolfia serpentina* Benth. Ex kurz root, *Coriandrum sativum* Linn. seed and *Piper nigrum* Linn. fruit. It is used in neurological disorders. **Methods and Material:** Plant parts were first cleaned, dried in the shade and powdered by passing through sieve # no. 80 and prepared as per the method described in UPI. SM formulation was evaluated using physicochemical tests: powder characterization, extractive value, alcohol, and water-soluble matter, Ash value, LOD at 105 ºC, pH and HPTLC fingerprinting. **Statistical Analysis Used:** Mean ± SEM. **Result:** Organoleptic characters of SM revealed brown color, characteristic odor, bitter taste, and moderately fine texture. Physicochemical parameters resulted in water-soluble extractive (9.58 ± 0.11), alcohol-soluble extractive (6.29 ± 0.07), total ash (5.87 ± 0.09), acid insoluble ash (3.09 ± 0.08), water-soluble ash (1.5 ± 0.05), LOD at 105 ºC (7.75 ± 0.058), pH of 1% and 10% solution were 6.4 ± 0.1 and 5.6 ± 0.25 respectively. The phytochemical analysis shows the presence of alkaloids, glycosides, tannins, flavonoids, steroids, terpenoids, carbohydrates, sugars, volatile oil, saponins, anthraquinones, proteins, and phenols. HPTLC fingerprinting data in two mobile phases n-Butanol: Acetic acid: water (5:1:4) and Toluene: Ethyl acetate: Formic acid (5:4:1) (v/v) was set in. **Conclusion:** standardization data of SM was obtained as a standard and for future reference.

INTRODUCTION: Standardization of herbal formulations is essential for the assessment of quality, purity, and efficacy of drugs. In recent years, plant-derived products are increasingly being sold out as medicinal products, nutraceuticals, and cosmetics and are available in health food shops and pharmacies or also as drugs prescribed in the non-allopathic systems [1].

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The quality evaluation of herbal formulations is of principal importance to justify their acceptability in the present world [2]. One of the major problems faced by the herbal industry is the unavailability of strict quality control profiles for herbal products and their formulations.

Regulatory bodies have laid down the standardization procedures and specifications for Unani preparations [3, 4]. There is a need to explore the medicinally important plants, and this can be achieved only if the herbal products are analyzed and evaluated by using sophisticated modern techniques of standardization. The World Health Organization (WHO) has cherished the importance of medicinal plants for public health care in

developing nations and has formulated guidelines to help the member states in their efforts to formulate national policies on non-conventional medicine and to study their probable usefulness including evaluation, safety, and efficacy [5]. It has become extremely important to make an effort towards standardization of the herbal formulation to be used as medicine.

METHODOLOGY:

Procurement of Raw Drugs: [6] Ingredients of *Safoof-e-Musakkin* was procured from the pharmacy of NIUM Kottigepalya Bangalore, Karnataka, India, and authentic shop. The identification of these drugs was done by the experts at National Institute of Unani Medicine Kottigepalya, Bangalore. The detail of the ingredients of *Safoof-e-Musakkin* is depicted in **Table 1.**

TABLE 1: INGREDIENTS OF *SAFOOF-E-MUSAKKIN*

S. no.	Name	Botanical name	Part used	Proportion
1	Bekh-e-Asrol	*Rauvolfia serpentina* Benth. Ex kurz	Root	44.64%
2	Kishneez Khusk	*Coriandrum sativum* Linn.	Seed	44.64%
3	Filfil Siyah	*Piper nigrum* Linn.	Fruit	10.71%

Preparation of Formulation: [6] All the drugs were first cleaned and dried in the shade and powdered by passing through sieve # no. 80. The formulation **Fig. 1** was prepared as per the method described in the National Formulary of Unani Medicines.

FIG. 1: *SAFOOF-E-MUSAKKIN* (SM)

Organoleptic Evaluation: [4] The Organoleptic evaluations refer to the evaluation of the *Safoof-e-Musakkin* formulation by color, odor, taste, appearance, particle size and texture.

Powder Characterization: [7, 8, 9]

Angle of Repose: The angle of repose indicates the flowability of the substance. The 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.

Bulk Density and Tapped Density: The weighed quantity (20 gm) of *Safoof-e-Musakkin* 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 flowability 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 the following equation.

$$\text{Carr's index (\% compressibility)} = 100 \times (1 - Db / Dt)$$

Where Db = Bulk density, Dt = Tapped density.

Hausner's Ratio: Hausner ratio has also been used as an indirect method of quantifying powder flow ability from bulk density.

$$\text{Hausner ratio} = Dt/ Db$$

Where Db = Bulk density and Dt =Tapped density. All the experiments were repeated in triplicate.

Physico-chemical Evaluation: The Physico-chemical evaluation of prepared *Safoof-e-Musakkin* was done by testing loss of weight on drying at 105ºC, total ash, acid insoluble ash, water soluble ash, pH of 1% and pH of 10% solution and extractive values.

Loss on drying at 105 ºC: [10] An accurately weighed 3 g of *Safoof-e-Musakkin* was taken in a petri dish. The crude drug was heated at 105 ºC in an oven till a constant weight and percentage moisture content of the sample was calculated concerning the weighed *Safoof-e-Musakkin* sample.

Ash Values: Determination of total ash, acid insoluble ash, and water soluble ash is done as per protocol for testing of ASU drug and UPI [4, 10].

Determination of pH: [11] 1% and 10% solution of *Safoof-e-Musakkin* was prepared in distilled water (w/v), and pH was determined by using digital pH meter.

Extractive Values: [10] Water soluble extractives: Five grams of *Safoof-e-Musakkin* 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 milliliters of the filtrate was evaporated to dryness in the petri dish, dried at 105 °C, and weighed. Percentage of water-soluble extractive concerning air-dried material was calculated.

Alcohol Soluble Extractives: Five grams of *Safoof-e-Musakkin* 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 milliliters of the filtrate was evaporated to dryness in a petri-dish, dried at 105 °C, and weighed. Percentage of alcohol-soluble extractive was calculated concerning the air-dried drug.

Successive Extractive Value and Non-Successive Extractive Value: [12]

Successive Extractive Value: The coarse powder of *Safoof-e-Musakkin* 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 a water bath. The extractive values were determined concerning 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-Musakkin* 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 evaporate on the water bath. Extractive values were determined concerning a drug is taken (w/w).

Qualitative Estimation: [11] Qualitativeestimations *Safoof-e-Musakkin* for organic constituent's *viz*. alkaloids, glycosides, tannins, flavonoids, sugars, saponins, phenols, proteins, resins, and steroids was done.

HPTLC Fingerprinting Analysis: The weighed quantity (5 g) of *Safoof-e-Musakkin* was extracted in a Soxhlet apparatus for 6 h using 100 ml of solvent (methanol) at a controlled temperature. HPTLC was performed on 20 cm × 10 cm aluminum-backed plates coated with silica gel 60F254 (Merck, Mumbai, India). Standard solution of sample solution was applied to the plates as bands by use of a Camag (Muttenz, 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 [13, 14].

RESULTS: The organoleptic properties of *Safoof-e-Musakkin* showed brown color, characteristic odor, bitter taste, fine texture, and particle size was no 80# sieve. The powder characterization of *Safoof-e-Musakkin* is depicted in **Table 2**. The physicochemical evaluation of *Safoof-e-Musakkin* is mentioned in **Table 3** and water, and alcohol soluble extractive values were found to be 9.58 ± 0.11 (% w/w) and 6.29 ± 0.07 (% w/w) respectively.

Successive and non - Successive extraction, Phytochemical Screening and HPTLC of *Safoof-e-Musakkin* are depicted in **Table 4-5** and **Table 6- 9** respectively **Fig. 2-11**.

TABLE 2: POWDER CHARACTERIZATION OF *SAFOOF-E-MUSAKKIN*

S. no.	Parameters	Percentage mean (n=3) ± SD
1	Bulk Density (gm/ml)	0.2702 ± 0.0033
2	Tapped Density (gm/ml)	0.3773 ± 0.0026
3	Carr's index	28.378 ± 0.325
4	Hausner's Ratio	1.396 ± 0.007
5	Angle of Repose	38.83^{0} ± 0.86

TABLE 3: PHYSICOCHEMICAL CHARACTERISTICS OF *SAFOOF-E-MUSAKKIN*

S. no.	Parameters	Percentage mean (n=3) ± SD
1	Loss on drying (%)	7.75 ± 0.058
2	Ash Content	
A	Total Ash (% w/w)	5.87 ± 0.09
B	Water soluble Ash (% w/w)	1.5 ± 0.05
C	Acid Insoluble Ash (% w/w)	3.09 ± 0.08
3	pH	
A	pH (1%)	6.4 ± 0.1
B	pH (10%)	5.6 ± 0.25

TABLE 4: SUCCESSIVE EXTRACTION AND NON-SUCCESSIVE EXTRACTION OF *SAFOOF-E-MUSAKKIN*

	Successive extractive value (%w/w)				Non-Successive extractive value(%w/w)				
Mean ± SEM	Petroleum ether	Benzene	chloroform	Ethanol	Petroleum ether	Benzene	chloroform	Ethanol	Water
	11.88 ± 0.12	1.33 ± 0.04	1.43 ± 0.1	2.29 ± 0.07	11.85 ± 0.1	12.6 ± 0.08	11.26 ± 0.18	18.25 ± 0.13	17.53 ± 0.09

TABLE 5: PHYTOCHEMICAL SCREENING (QUALITATIVE ESTIMATION) OF *SAFOOF-E-MUSAKKIN*

S. no.	Parameters	Result
1	Alkaloids	+
2	Glycosides	+
3	Tannins	+
4	Steroids	+
5	Flavonoids	+
6	Sugars	+
7	Phenols	+
8	Proteins	+
9	Saponins	+
10	Terpenoids	+
11	Anthraquinones	+
12	Essential oils	+
13	Fatty acids	+

TABLE 6: R_f VALUE, NO. OF PEAKS, PEAK AREA, AND HEIGHT OF *SAFOOF-E-MUSAKKIN* IN N-BUTANOL: ACETIC ACID: WATER AT 254nm

Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.23	0.1	0.30	82.1	6.49	0.32	58.5	2971.0	10.20
2	0.32	59.7	0.34	318.3	25.18	0.36	126.7	5098.3	17.50
3	0.36	128.7	0.37	151.1	11.95	0.39	55.2	2447.2	8.40
4	0.39	55.7	0.42	310.5	24.55	0.47	67.8	9858.4	33.84
5	0.47	68.8	0.49	105.4	8.33	0.51	63.1	2211.3	7.59
6	0.51	63.5	0.53	94.3	7.46	0.55	40.7	2065.4	7.09
7	0.55	41.6	0.58	202.8	16.04	0.61	1.9	4477.7	15.37

TABLE 7: *Rf* VALUE, NO. OF PEAKS, PEAK AREA, AND HEIGHT OF *SAFOOF-E-MUSAKKIN* IN N-BUTANOL: ACETIC ACID: WATER AT 366 nm

Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.08	1.7	0.10	143.3	7.42	0.12	100.7	2429.4	3.98
2	0.12	101.5	0.15	120.7	6.25	0.17	96.2	3956.2	6.49

3	0.18	96.5	0.21	119.6	6.19	0.22	116.8	3449.1	5.65
4	0.23	117.0	0.27	205.7	10.65	0.28	195.2	5809.7	9.52
5	0.28	195.6	0.32	373.8	19.36	0.34	238.4	13327.6	21.85
6	0.34	239.2	0.36	419.4	21.72	0.38	245.6	9485.7	15.55
7	0.38	246.9	0.43	447.5	23.18	0.50	23.9	19679.7	32.28
8	0.51	24.4	0.54	100.8	5.22	0.58	9.1	2867.2	4.70

TABLE 8: *Rf* VALUE, NO. OF PEAKS, PEAK AREA, AND HEIGHT OF *SAFOOF-E-MUSAKKIN* IN TOLUENE: ETHYL ACETATE: FORMIC ACID AT 254 nm

Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.06	2.7	0.08	67.1	3.46	0.11	1.0	1098.8	1.99
2	0.12	1.1	0.13	28.1	1.45	0.16	0.5	478.8	0.87
3	0.16	0.8	0.19	210.7	10.87	0.23	0.1	4257.4	7.70
4	0.45	3.3	0.47	14.7	0.76	0.48	12.0	241.5	0.44
5	0.48	12.3	0.50	55.1	2.84	0.53	13.2	1099.2	1.99
6	0.53	13.5	0.57	207.9	10.73	0.58	197.6	3734.1	6.75
7	0.58	198.6	0.59	209.0	10.79	0.60	195.0	2232.7	4.04
8	0.60	195.2	0.63	577.5	29.80	0.68	209.9	23145.5	41.87
9	0.68	211.1	0.71	273.4	14.11	0.73	241.5	9099.0	16.46
10	0.73	241.5	0.76	294.7	15.21	0.81	2.4	9896.4	17.90

TABLE 9: *Rf* VALUE, NO. OF PEAKS, PEAK AREA, AND HEIGHT OF *SAFOOF-E-MUSAKKIN* IN TOLUENE: ETHYL ACETATE: FORMIC ACID AT 366 nm

Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.47	0.6	0.51	44.2	4.18	0.53	11.8	906.1	2.66
2	0.53	12.0	0.57	295.6	27.97	0.59	86.3	5709.7	16.77
3	0.59	89.1	0.63	716.9	67.84	0.72	15.7	27435.4	80.57

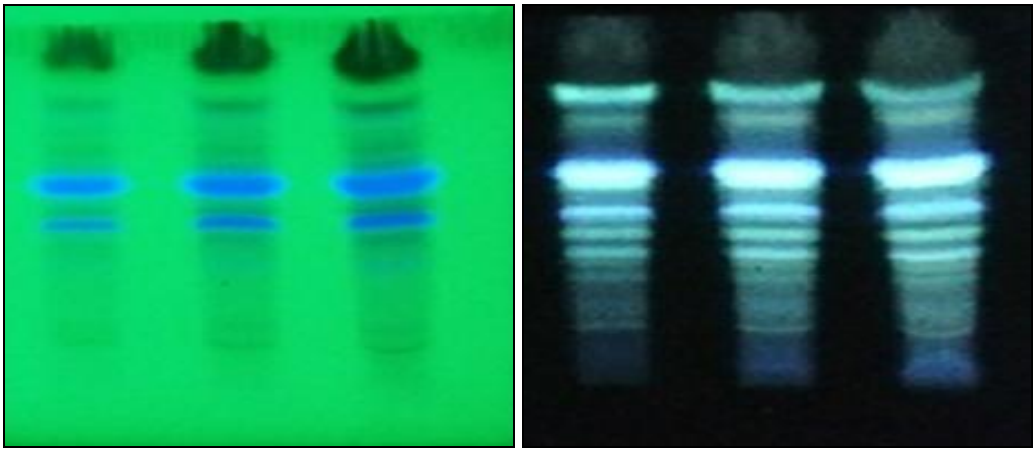

FIG. 2: HPTLC PHOTOS OF *SAFOOF-E-MUSAKKIN* (SM) METHANOLIC EXTRACT IN N BUTANOL: ACETIC ACID: WATER AT 254 nm AND 366 nm

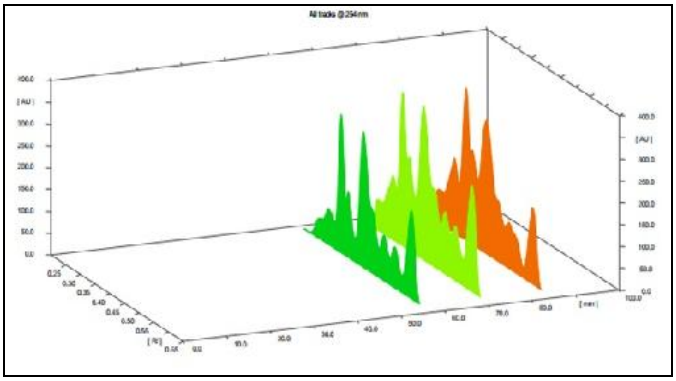

FIG. 3: HPTLC 3-D DENSITOMETRIC SCAN OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN N-BUTANOL: ACETIC ACID: WATER AT 254 nm

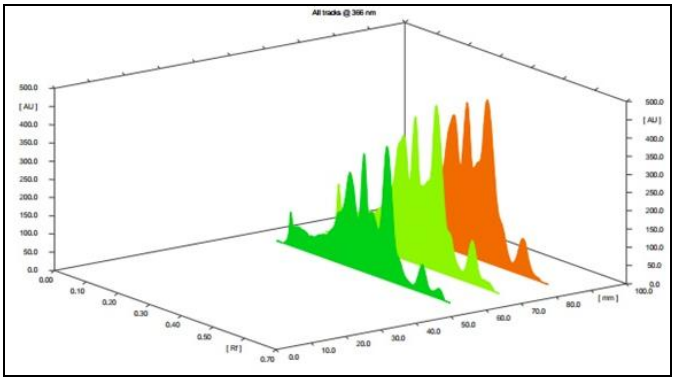

FIG. 4: HPTLC 3-D DENSITOMETRIC SCAN OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN N- BUTANOL: ACETIC ACID: WATER AT 366 nm

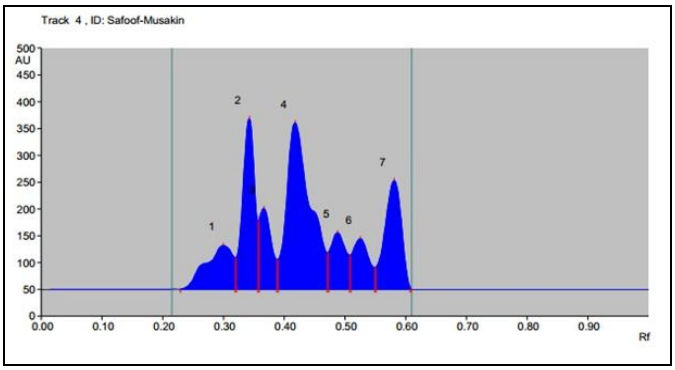

FIG. 5: HPTLC FINGERPRINT PROFILE OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN N-BUTANOL: ACETIC ACID: WATER AT 254 nm

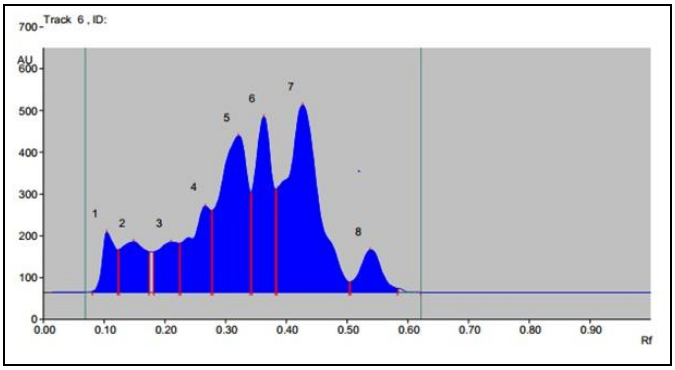

FIG. 6: HPTLC FINGERPRINT PROFILE OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN N-BUTANOL: ACETIC ACID: WATER AT 366 nm

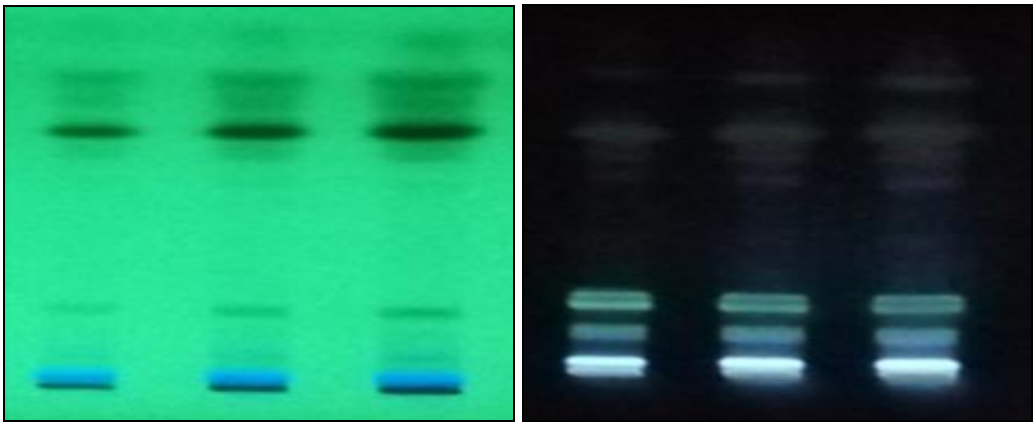

FIG. 7: HPTLC PHOTOS OF *SAFOOF-E-MUSAKKIN* (SM) METHANOLIC EXTRACT IN TOLUENE: ETHYL ACETATE: FORMIC ACID AT 254 nm AND 366 nm

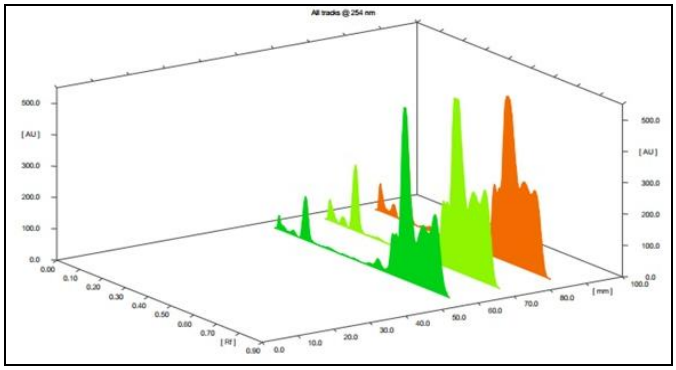

FIG. 8: HPTLC 3-D DENSITOMETRIC SCAN OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN TOLUENE: ETHYL ACETATE: FORMIC ACID AT 254 nm

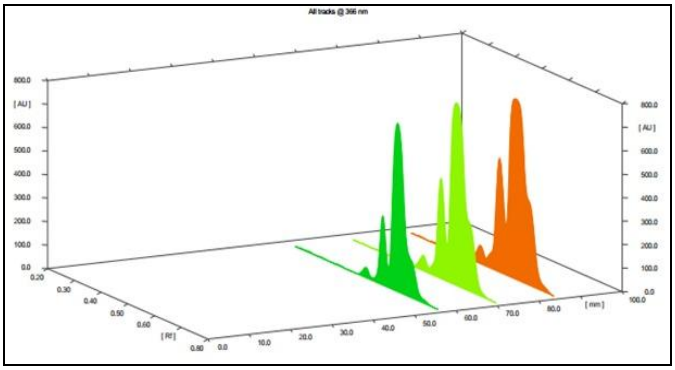

FIG. 9: HPTLC 3-D DENSITOMETRIC SCAN OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN TOLUENE: ETHYL ACETATE: FORMIC ACID AT 366 nm

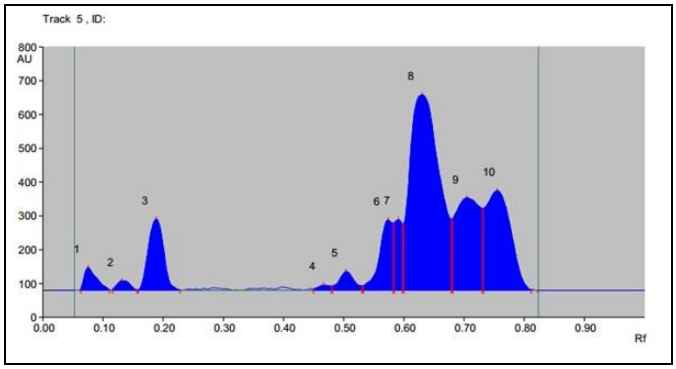

FIG. 10: HPTLC FINGERPRINT PROFILE OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN TOLUENE: ETHYL ACETATE: FORMIC ACID AT 254 nm

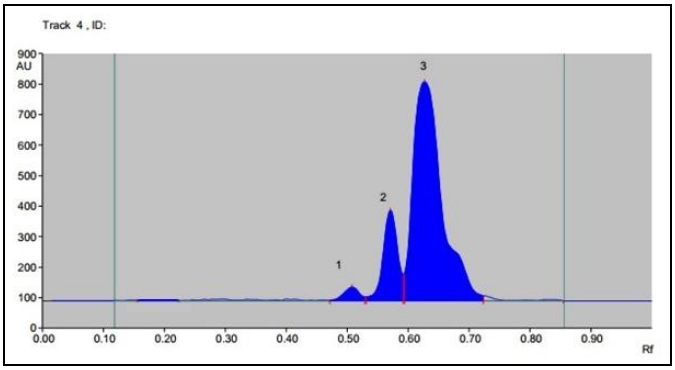

FIG. 11: HPTLC FINGERPRINT PROFILE OF METHANOLIC EXTRACT OF *SAFOOF-E-MUSAKKIN* (SM) IN TOLUENE: ETHYL ACETATE: FORMIC ACID AT 366 nm

DISCUSSION: Finished product of *Safoof-e-Musakkin* was brown as per color chart (No. PMS 1385 of Panton color chart), [15] bitter in taste, aromatic odor and without any clumping and aggregation. Organoleptic characteristic of the formulation was documented. The mean values of bulk density, tapped density, angle of repose, Hausner's ratio and compressibility index were 0.2702 ± 0.0033, 0.3773 ± 0.0026, 38.83^{0} ± 0.86, 1.396 ± 0.007 and 28.378 ± 0.325 respectively. Hausner's ratio and compressibility index are the simple and popular method to determine the flow characteristics of the powder. The flow characteristics of powder depending on the size, shape and size distribution of particles [16]. The compressibility index of *Safoof-e-Musakkin* lies between 23-35 according to the scale of flowability; it shows the SM has a poor flow character. The Hausner's ratio of *Safoof-e-Musakkin* lies in between 1.25-1.5, it indicates moderate flowability [17, 18]. **Table 2** Angle of repose displayed Passable flow property [18]. Powder characterization parameter was set in. The mean percentage of loss of weight on drying of *Safoof-e-Musakkin* was 7.75 ± 0.058.

Table 3 It is mentioned that the water content in plant drugs can vary between 8% and 14%. The presence of an excessive amount of moisture in plant drugs causes hydrolysis of constituents, growth of bacteria and fungi and biochemical reactions. The pharmacopoeial monographs compulsorily limit the water content, especially in drugs that have hygroscopic nature, or in which the excessive amounts of water causes deterioration of products [19]. As finished *Safoof-e-Musakkin* contains less amount of moisture, it can be expected that it will be stable/safe for a 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 *Safoof-e-Musakkin* was found to be 5.87 ± 0.09% w/w. These values were found to be 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 *Safoof-e-Musakkin* were 3.09 ± 0.08% w/w and 1.5 ± 0.05% w/w respectively [20]. **Table 3** pH of *Safoof-e-Musakkin* in 1% solution was 6.4 ± 0.1 while the pH of 10% solution was 5.6 ± 0.25. **Table 3** It is slightly acidic because two ingredients of this formulation contain a volatile oil which was responsible for acidic nature [21]. The correlation between the pH and microbial contamination was studied by Abba *et al*., and suggested that a neutral or alkaline pH favors high microbial contamination levels of the herbal preparations [22]. The mean percentage of water-soluble and alcohol-soluble extractive values of *Safoof-e-Musakkin* were 9.58 ± 0.11 and 6.29 ± 0.07 respectively. The mean percentage of successive extractive values in petroleum ether, benzene, chloroform, and ethyl alcohol were 11.88 ± 0.12, 1.33 ± 0.04, 1.43 ± 0.1 and 2.29 ± 0.07 respectively. Non-successive extractive values in petroleum ether, benzene, chloroform, ethyl alcohol and water were 11.85 ± 0.1, 12.6 ± 0.08, 11.26 ± 0.18, 18.25 ± 0.13 and 17.53 ± 0.09 respectively.

Table 4 Extractive value of a drug in a 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 [23]. High value was noted in petroleum ether, alcohol, and aqueous extract because of *safoof* ingredient such as *Rauwolfia serpentina* Benth. Ex kurz contains many important alkaloids, such as reserpine, serpentinine, and ajmalicine; [24] *Coriandrum sativum* Linn. contains Essential oil (coriandroal) [25] and *Piper nigrum* Linn. contains essential oil [25]. Organic constituent's *viz*. alkaloids, glycosides, tannins, flavonoids, sugars, saponins, phenols, proteins, steroids, *etc*. were qualitatively estimated **Table 7**.

HPTLC: HPTLC plates of methanolic extract of *Safoof-e-Musakkin* 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 a peak height of *Safoof-e-Musakkin* in two separate mobile phases viz. n-Butanol: Acetic acid:

water (5:1:4) **Fig 2-6** and Toluene: Ethyl acetate: Formic acid (5:4:1) **Fig. 7-11.** Both the phases were analyzed under 254 nm, 366 nm respectively. Area percentage of peak no. 4 of *Safoof-e-Musakkin* analyzed under 254 nm in n-Butanol: Acetic acid: water (5:1:4) was highest (33.84%). **Table 6** Area percentage of peak no. 7 of *Safoof-e-Musakin* analyzed under 366 nm in n-Butanol: Acetic acid: water (5:1:4) was highest (32.26%). **Table 7** Area percentage of peak no. 8 of *Safoof-e-Musakkin* analyzed under 254 nm in Toluene: Ethyl acetate: Formic acid (5:4:1) was highest (41.87%).

Table 8 Area percentage of peak no. 3 of *Safoof-e-Musakkin* analyzed under 366nm in Toluene: Ethyl acetate: Formic acid (5:4:1) was highest (80.57%). **Table 9** Further studies can also be done by the help of standards and quantitative estimation and identification of the ingredients. Present HPTLC fingerprinting data can help in the authentication and identification of *Safoof-e-Musakkin* in the performed solvent system and extract.

Reported activity on the constituent of SM reveals important pharmacological activity owing to their uses mention in Unani text. The reported activity of methanolic root extract of *Rauwolfia serpentina* Benth. Ex kurz are antidiabetic, antiatherogenic, and cardioprotective activities tested *in-vivo*; [26] the aqueous extract shows antifungal activity tested i*n-vitro* [27]. The reported activity of hydroalcoholic extract of *Coriandrum sativum* Linn. reveals anti-anxiety activity tested *in-vivo*;[28] the hydroalcoholic extract shows neuroprotective activity tested in-vivo.[29] Reported activity of pet. ether extract of *Piper nigrum* Linn. reveals anti-oxidant activity tested *in-vitro* [30]. These reported activities can be correlated with the constituent of the ingredients detected in the formulation. Standardization is an important measure for knowing the quality, purity and for sample identification. It is one of the simplest and cheapest methods for the correct identity of the materials. These are the preliminary standards of the formulation SM, and further sophisticated standards can also be developed with a quantitative estimation of the ingredients and other parameters in the future.

CONCLUSION: The prepared powder formulation *Safoof-e-Musakkin* was screened for various standardization parameters as per Unani pharmacopoeial standards and its physicochemical standards including HPTLC was set in. The research outcome of the standardization parameters may be used as a standard monograph for identification and further evaluation or future research work.

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