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PHYSICOCHEMICAL STUDY AND DEVELOPMENT OF SOPs OF A POLYHERBAL UNANI FORMULATION: SAFŪF-I MU'ALLIF

Abrar Alam *, Javed Inam Siddiqui and Munawwar Husain Kazmi

Department of Ilmul Advia (Pharmacology), Central Research Institute of Unani Medicine, Hyderabad - 500038, Telangana, India.

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Correspondence to Author:
Abrar Alam

P.G. Scholar,
Department of Ilmul Advia (Pharmacology), Central Research Institute of Unani Medicine, Hyderabad - 500038, Telangana, India.

E-mail: abraralamcrium@gmail.com

ABSTRACT: In the Unani system of medicine, different dosage forms of the drug are used; out of them powder dosage form is known as Safūf, which is an important dosage form and has been used frequently since long. There are many Unani preparations used in the form of Safūf to treat various diseases. Safūf –I Mu'allif (SM) is a polyherbal Unani formulation that is known to be used as Spermatogenic agent and semen thickening agent. The drug is also used to treat sexual disorders like premature ejaculation, nocturnal emission, sexual debility, *etc.* Physicochemical study and development of standard operating procedures (SOPs) of polyherbal Unani formulations are essential to assess the quality, purity, and efficacy and consistency of drugs. The study formulation consists of following ingredients Tāl Makhānā (*Asteracantha longifolia* Nees.), Sā'lab Miṣri (*Orchis latiflolia* L.), Singhārā Khushk (*Trapa bispinosa* Roxb.), Gond Kīkar (*Acacia Arabica* Willd.), Māzu Sabz (*Quercus infectoria* Oliv.), Maṣṭagi Rumi (*Pistacia lentiscus* L.), Nishāshtah Gandum (*Triticum aestivum* L), Shaker Sūfed (*Saccharum officinarum* L.). The main aim of this study was to standardize the formulation on widely accepted physicochemical parameters and also develop the standard operating procedures (SOPs) for the preparation of the formulation scientifically.

INTRODUCTION: The Unani System of Medicine, as its name suggests, originated in Greece (Yūnān) and Buqrāt (Hippocrates, 460-370 BC), established the ground for Medicine to develop it as a systematic science and a Roman scholar Jalinoos (Galen) was lay down the foundation of this science on which Arab and Persian scholars and physicians like Rabbanṭabarī (775-890 AD), al-Rāzī (865-925 AD) and Ibn Sīnā (980-1037 AD) elaborate the medical science based on the context of the education and provide the way that the Unani System of Medicine to reach the great heights.

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Ever since that time the Unani system of Medicine (USM) has been recognized as Greco-Arab Medicine. In India Unani System of Medicine introduced by Arabs and Iran and made a long journey to institute itself as one of the favorite medical systems in the country. However, alike other traditional systems of medicine, the drug of the Unani system of medicine is obtained from natural sources.

During the few eras, there has been an ever-increasing demand for drugs from natural sources, exclusively from both developing and developed countries [1]. This revival of interest is developed mainly due to the current widespread belief that USM is safe, effective, and more truthful. Up to 65% of the population in India and 80% in Africa depend on traditional medicine to help to meet their health care needs [2]. Physicochemical study and development of standard operating procedures (SOPs) of Unani formulations are essential to

assess the quality, purity, and efficacy of drugs. The scientific validation of herbal formulations is of great importance to justify their acceptability in a contemporary period of medicine [3]. 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 including evaluation, safety and efficacy [4, 5]. There are a marvelous role and scope in Indian traditional medicine like Ayurveda, Unani, Yoga and Naturopathy, Siddha, and Homeopathy for the production of standardized and therapeutically effective formulations. There is a need to explore the medicinally important plants and their formulations. Quality evaluation and development of SOPs of herbal preparation are a fundamental requirement of the pharmaceutical industry and other organizations dealing with Unani and herbal products for wide acceptability [5].

The application of Good Manufacturing Practices (GMP) in the manufacturing of Unani medicines is an essential tool to assure their quality. The concept of SOPs is a part of GMP, and it is commonly used in the pharmaceutical industry. SOPs is an authorized written procedure giving instructions for performing operations not necessarily specific to a given product or material (*e.g.,* equipment operation, maintenance, and cleaning; validation; cleaning of premises and environmental control; sampling and inspection). The USM consists of a large number of traditional formulations and used them since, a long with for the prevention and cure, the different ailments in India and overseas. This USM used different types of formulations like Safūf (Powder), Itrifal (Semisolid), Mājoon (Semisolid), Jawārish (Semisolid), Arq (Distillate), Qurs (Tablets), and Habb (Pills), *etc*. Although of their potency, they have been widely criticized due to lack of physicochemical studies and development of SOPs [6-10]. Safūf-i Mu'allif (SM) is a polyherbal powder formulation used in the Unani System of Medicine, which is known to be used as Spermatogenic agent and semen thickening agent. The drug is also used to treat sexual disorders like premature ejaculation, nocturnal emission, sexual debility, *etc*. since ancient times and the formulation is mentioned in Qarabadin–IMajidi (A Classical Unani Pharmacopoeia) [11].

This formulation is not evaluated for its physicochemical standardization and not develops SOPs since yet, as revealed by the literature survey. Thus keeping this goal in mind, the aim of the present research work was carried out to develop the quality control standards of SM with using sophisticated modern analytical techniques based on physicochemical parameters and also develop the SOPs related to the preparation of formulation.

MATERIALS AND METHODS: Plant Ingredients of the formulation SM were procured from the GMP certified pharmacy and identified and authenticated them by the Pharmacognosist at the quality testing laboratory of Central Research Institute of Unani Medicine (CRIUM), Hyderabad, India.

Preparation of Formulation: The study formulation Safūf-i Mu'allif has been prepared at Ilmul Advia (Drug quality testing Laboratory) as per prescribed procedure mentioned in Qarābadīn – I Majidi [11] (Classical Unani Pharmacopoeia), the Unani Pharmacopoeia of India (UPI) [12] and National Formulary of Unani Medicine (NFUM)[13].

Foreign Matter in Crude Drugs: The ingredients of the crude drugs Tāl Makhānā (*Asteracantha longifolia* Nees.), Sa'lab Miṣri (*Orchis latiflolia* L.), Singhārā Khushk (*Trapa bispinosa* Roxb.), Gond Kīkar (*Acacia arabica* Willd.), Māzu Sabz (*Quercus infectoria* Oliv.), Maṣṭagi Rumi (*Pistacia lentiscus* L.), Nishāshtah Gandum (*Triticum aestivum* L), Shaker Sūfed (*Saccharum officinarum* L.) were inspected with naked eye and magnifying glass and examined for the presence of any foreign matter and adulterants.

Development of Standard Operating Procedures (SOPs): The study formulation Safūf-i Mu'allif was prepared according to its composition as per Qarabadin –IMajidi, UPI, NFUM and the following SOPs developed in accordance with the preparation [11, 12, 13].

TABLE 1: THE COMPOSITION OF THE STUDY FORMULATION

SAFŪF-I-MU'ALLIF (Form: Powder)				
S. no.	**Unani name of ingredients**	**Botanical name/ English name**	**Part used**	**Quantity (in-grams)**
1	Tāl Makhānā	*Asteracantha longifolia* Nees.	Seed	100
2	Sa'lab Miṣri	*Orchis latiflolia* L.	Root	80
3	Singhārā Khushk	*Trapa bispinosa* Roxb.	Dried fruit	150
4	Gond Kīkar	*Acacia Arabica* Willd.	Gum	150
5	Māzu Sabz	*Quercus infectoria* Oliv.	Galls	75
6	Maṣṭagi Rumi	*Pistacialentiscus* L.	Oleo-gum resin	75
7	Nishāshtah Gandum	*Triticum aestivum* L.	Starch Powder	100
8	Shaker Sūfed	*Saccharum officinarum* L.	Crystals	730

- Take all the ingredients of pharmacopoeial quality.
- Clean the ingredients thoroughly for the removal of foreign matter.
- All the ingredients of the study formulation were grinded and powdered separately except Mastagi Rumi and Gond Kikar.
- The powdered ingredients were separately passed through sieve no. 80.
- Mastagi Rumi was powdered with gently applied hand pressure with the help of stone, mortar, and pestle.
- Gond Kīkar was kept in the oven at 40-50 ºC temperature to remove the moisture; then, it was grinded and sieved through mesh no.80.
- All the powdered ingredients were gradually mixed together to obtain a homogenous mixture.
- Finally, at last the powdered Shaker Sūfed was added uniformly in the mixture and obtained the formulation.
- The obtain formulation was kept in moisture free glass airtight container away from sunlight and moisture.
- The same method was adopted for the preparation of the formulation's three batches.

Physico-chemical Evaluation: The Physico-chemical evaluation of prepared SM was done by testing total ash, acid insoluble ash, water-soluble matter, alcohol soluble matter, Successive extractive value, pH of 1% and pH of 10% solution, loss of weight on drying at 105 ºC, Bulk density and fluorescence analysis of formulation's ingredients [14-24].

Ash Value Determinations: Apart from the doses form (Powder) of the study formulation, the determination of ash value is a significant and important parameter. It was analyzed by total ash and acid-insoluble ash parameter. The main aim for the determination of ash value of any drugs is usually to remove all traces of organic matter and detection of inorganic substances like silica, magnesium, *etc*.

Total Ash: The total ash is to measure the total amount of material remaining after ignition. Unani Pharmacopoeia of India and WHO prescribe suitable methods for the determination of ash values. About 5 g of SM was placed in the silica crucible. The powdered drug was spread as a fine layer at the crucible base and ignited on the burner; after that, it was incinerated at a temperature not exceeding 450 °C until free from carbon.

The crucible was cooled and weighed to get the total ash content. The percentage of total ash was calculated with reference to the amount of air-dried drug taken. This procedure was triplicated. The mean value and standard deviation were calculated.

Acid Insoluble Ash: Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. The total ash which obtained as described above, boil with 25 ml of dilute hydrochloric acid for 5 min. The insoluble matter was collected on ashless filter paper (Whatman filter paper no. 42).

Then ashless filter paper was transferred in tarred silica crucible and ignited at a temperature not exceeding 450 ºC to a constant weight, and the percentage of acid-insoluble ash was calculated with reference to the amount of air-dried drug has

been taken. This procedure was repeated for three times. The mean value and standard deviation were calculated.

Alcohol-Soluble Matter: 5 g weight of Safūf-i Mu'allif was taken in a glass stoppered conical flask separately and macerated with 100 ml of Ethyl alcohol of the specified strength in a closed flask for 24 h. It was shaken frequently during 6 h and allow to stand for 18 h.

Filtered rapidly, through dry filter paper taking precautions against loss of the solvent, 25 ml of the filtrate was transferred in a tared Petri-dish and evaporated to dryness on water-bath. Then dried at 105 °C, cooled in desiccators, and weighed instantly.

Calculate the percentage of alcohol-soluble matter with reference to the air-dried drug taken. This procedure was repeated three times. The mean value and standard deviation of both the samples were calculated.

Note: Follow the same procedure of alcohol-soluble extractive excepting the solvent (alcohol), which is replaced by water for the determination of the percentage of water-soluble matter.

Successive Extractive Value: The successive extractive values of the study formulation were carried out with the help of Soxhlet's apparatus with different non-polar and polar solvents system viz. Petroleum ether 40-60 ºC, chloroform, ethyl acetate, ethanol, and acetone. The required quantity of the study drug powder was taken and subjected to successive extraction with a suitable solvent. That was started with a non-polar solvent followed by increasing the polarity of the solvent *viz.* petroleum ether 40-60 ºC, chloroform, ethyl acetate, ethanol, and acetone.

The process of extraction continues for 24 h or till the solvent in the siphon tube becomes colorless. After extraction, the dilute extracts were taken in a tared flat dish and evaporated to get solid residue on the water bath and then weight it to the obtained constant weight of the extract. The extract values were determined with reference to the weight of air-dried drug (% w/w). The procedure was repeated three times, and the mean value for each solvent extract was calculated.

Determination of pH: pH of freshly prepared 1% w/v solution and 10% w/v solution in 100 ml distilled water of the sample was determined using digital pH meter (make digisun) separately.

Loss of Weight on Drying at 105 ºC: The amount of moisture, as well as volatile components present in a particular sample, was determined by this parameter.

About 5 g of the powdered drug sample was placed on a tared flat evaporating dish and dried at 105 °C in the oven for 6-8 h.

Then the dish was removed from the oven, cover it promptly, and allow cooling at room temperature and weighed. The drying was continued until two consecutive readings and constant weight to be obtained between two successive weighing and not varying more than 0.25% or 5 mg unless otherwise stated in the test procedure.

Bulk Density: 10 gm of Safūf-i Mu'allif was filled into a graduated cylinder with the help of a funnel and that is carefully added into the cylinder with the help of a spoon without any loss.

The initial (bulk) volume was noted, and the samples were then tapped until no further reduction in volume was noted. This procedure was repeated and triplicate in three batches of formulation-

Bulk Density = Mass (drug wt.) / Bulk volume

Fluorescence Analysis of Formulation's Ingredients: Take a small quantity of each dried powder ingredient of the formulation and placed it into dried test tubes.

Added 5 ml of different organic solvents like distilled water, ethanol, sulphuric acid, Sodium hydra oxide, separately to the test tubes.

Then, all the tubes were shaken, and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible daylight and UV light of short wavelength (254 nm) and UV light of long-wavelength (365 nm) for their color fluorescence analysis.

The color observed by the application of different reagents in various radiations was recorded [25-28].

TABLE 2: FLUORESCENCE ANALYSIS OF INGREDIENTS OF SAFŪF-I MU'ALLIF

S. no.	Experiments	Crude ingredients of the formulation	Visible Daylight	UV Light	
				254 nm (Short wavelength)	365 nm (long wavelength)
1	Powder as such	Sā'labMiṣri	Yellow	Brownish-yellow	Yellowish green
		SinghārāKhushk	White	Whitish brown	White
		GondKīkar	Whitish yellow	Yellowish-brown	Yellowish green
		MāzuSabz.	Off white	Greyish yellow	Green
		Maṣṭagi Rumi	Light yellow	Off white	Green
2	Powder + Distil water	Sā'labMiṣri	Light yellow	Off white	Light yellow
		SinghārāKhushk	White	White	White
		GondKīkar	Off white	Brown	Green
		MāzuSabz	Light yellow	Dark gray	Gray
		Maṣṭagi Rumi	Light yellow	Light green	Green
3.	Powder + Ethanol	Sā'labMiṣri	Light yellow	Off white	Light yellow
		SinghārāKhushk	White	White	Greyish white
		GondKīkar	Colourless	Gray	Light green
		MāzuSabz	Yellowish	Light grey	Green
		Maṣṭagi Rumi	Light yellow	Light green	Greenish
4	Powder + Conc. H_2SO_4	Sā'labMiṣri	Black	Black	Black
		SinghārāKhushk	Black	Brownish black	Black
		GondKīkar	Yellowish-brown	Brown	Green
		MāzuSabz	Yellowish-brown	Greenish brown	Light green
		Maṣṭagi Rumi	Brick red	Blackish brown	Dark brown
5	Powder + 50% H_2SO_4	Sā'labMiṣri	Black	Black	Black
		SinghārāKhushk	Reddish-brown	Blackish brown	Yellowish green
		GondKīkar	Blackish brown	Black	Brownish green
		MāzuSabz	Brownish black	Black	Greenish black
		MaṣṭagiRumi	Light brown	Yellowish-brown	Green
6	Powder + 10% NaOH	Sā'labMiṣri	Yellow-brown	Colourless	Light green
		SinghārāKhushk	Light yellow	Dark brown	Green
		GondKīkar	Colourless	Colourless	Colorless
		MāzuSabz	Yellowish-brown	Dark brown	Green
		Maṣṭagi Rumi	Light yellow	Light green	Green

TABLE 3: PHYSICOCHEMICALEVALUATION OF SAFŪF-I MU'ALLIF

S. no.	Parameters	Percentage mean ± S.D. (In range)
1	Total ash (% w/w)	0.8958 ± 0.05 - 0.8984 ± 0.08
2	Acid insoluble ash (% w/w)	0.0699 ± 0.06 - 0.1006% ± 0.09
3	Water soluble matter (% w/w)	87.9099 ± 0.44 - 88.7351 ± 0.33
4	Alcohol soluble matter (% w/w)	14.6981 ± 0.23- 15.7252 ± 0.64
5	pH (1%) aqueous solution	5.12 ± 0.02- 5.15 ± 0.02
6	pH (10%) aqueous solution	5.15 ± 0.01- 5.17 ± 0.01
7	Loss of weight on drying at 105 ºC	2.7230 ± 0.05- 2.9296 ± 0.07
8	Bulk density	0.73%

TABLE 4: SUCCESSIVE EXTRACTIVES VALUES (% W/W) OF SAFŪF-I MU'ALLIF (THREE BATCHES) IN DIFFERENT POLAR AND NON-POLAR SOLVENTS

Successive extractives (% w/w)	Batch 1	Batch 2	Batch 3	Mean ± SD
Petroleum ether extract (40-60 ºC)	6.7394	7.5630	8.0284	7.4436 ± 0.65
Chloroform extract	2.7736	3.0999	2.6555	2.8430 ± 0.23
Ethyl acetate extract	1.1202	1.1752	0.7692	1.0215 ± 0.22
Ethanol extract	5.1200	5.8001	5.0112	5.3104 ± 0.43
Acetone extract	0.8826	0.8314	1.9161	1.2100 ± 0.61

RESULTS: The development SOPs of Safūf-i Mu'allif depicted in **Table 1**. The fluorescence analysis of the ingredients of the study is shown in **Table 2**. The physicochemical study of SM is mentioned in **Table 3**. Water and alcohol soluble matter values were found to the range of 14.6981 ± 0.23 to 15.7252 ± 0.64% and 87.9099 ± 0.44 to 88.7351 ± 0.33% respectively. Successive extractions of SM from non-polar to polar solvent are shown in **Table 4** and **Fig. 1,** respectively.

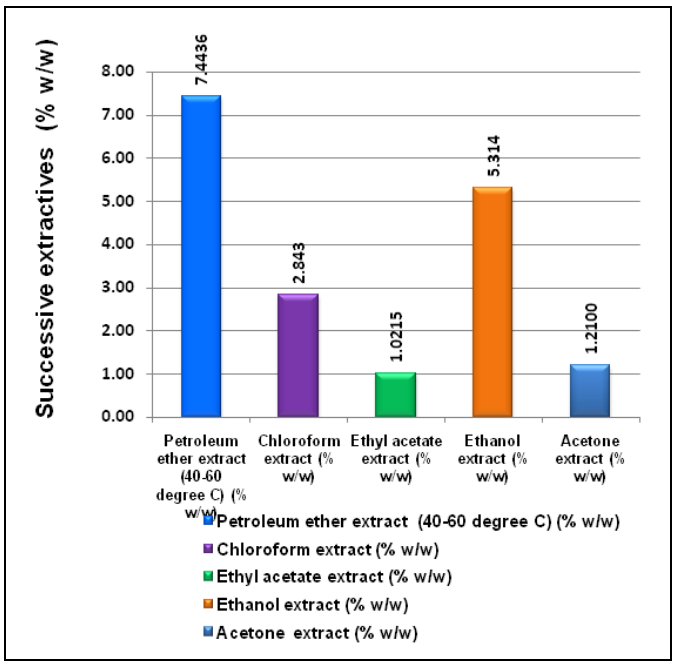

FIG. 1: SUCCESSIVE EXTRACTIVES VALUES (% w/w) OF SM (THREE BATCHES) IN DIFFERENT POLAR AND NON-POLAR SOLVENTS

DISCUSSION: Nature gives a great gift in the form of medicinal plant, and Herbal remedies are consuming by the people from the centuries for safety, purity, cultural acceptability, and lesser side effects. Plant and their products have been utilized with varying success to cure and prevent diseases, as these are considered a rich source of therapeutic agents throughout history. Due to fewer side effects of herbal products as compared to synthetic drugs, the acceptance of herbal products is increasing globally.

It is an assumption that the herbal formulations are considered harmless, and due to this concept, these are being consumed increasingly by people without any recommendation. However, some of them can cause health problems, some are ineffective, and some may interact with other drugs if they are not checked for this quality and safety. Thus, physicochemical studies and development of SOPs of polyherbal Unani formulations are essential in order to assess the quality and purity of drugs, based on the concentration and effectiveness of their active principles.

In this way, physicochemical studies and development of SOPs for Safūf-i Mu'allif' (SM) were generated in this work. Fluorescence is an important phenomenon displayed by various phytoconstituents present in plant materials.

Some natural products show fluorescence in the visible range in daylight and the natural product which does not visibly fluoresce in day light produces fluorescence in the ultraviolet light in a different wavelength. Some of the phytoconstituents may be often converted into fluorescent derivatives by using different chemical reagents and chemicals though they are not fluorescent, hence we can often assess qualitatively some crude drugs obtained from plants using fluorescence as it is the most important parameter of pharmacognostical as well as phytochemical evaluation. The results of the fluorescent analysis of the crude ingredients powder of SM were shown in **Table 2**, the herbal formulation showed characteristic colour fluorescence upon treatment with different chemical reagents.

The mean percentage values of the total ash (% w/w; Mean ± SD) and acid insoluble ash (% w/w; Mean ± SD)value were found to be in the range of 0.8958 ± 0.05 to 0.9231 ± 0.08 and 0.0699 ± 0.06 to 0.1006 ± 0.09 respectively. The amount of the extract that the drugs yield in a solvent is often an approximate measure of the amount of a certain constituent that the drug contains. Therefore, for establishing the standard of any drug, the extractive values play a major role.

The mean percentage of alcohol and water-soluble matter (% w/w; Mean ± SD) of SM was found to be in the range of 14.6981 ± 0.23 to 15.7252 ± 0.64 and 87.9099 ± 0.44 to 88.7351 ± 0.33 respectively. The mean percentages of the successive extractive values (% w/w; Mean ± SD) of Safūf-I Mu'allif were found to be 7.4436 ± 0.65, 2.8430 ± 0.23, 1.0215 ± 0.22, 5.3104 ± 0.43 and 1.2100 ± 0.61% with Petroleum ether extract (40-60 ºC), Chloroform extract, Ethyl acetate extract, Ethanol extract, and Acetone extract respectively. Thus it showed that the successive extractive value of the study formulation was maximum in petroleum ether. pH was determined in 1% and 10% aqueous solution of Safūf-I Mu'allif, and the values were found to be 5.1, which slightly acidic in nature.

The Loss of weight on drying the percentage of moisture and volatile matter is calculated. On the basis of LOD content gives an idea regarding the adulteration of the drug with respect to moisture. The LOD content of the drugs is variable because

most vegetable drugs are hygroscopic, and excessive moisture content becomes an ideal medium for the growth of the different types of bacteria as well as fungi. They subsequently spoil the purity of the drug, which can lead to the deterioration of the drugs. The mean percentage of loss of weight on drying at 105 °C (% w/w; Mean ± SD) was found to be 2.7230 ± 0.05 to 2.9296 ± 0.07 in SM.

The bulk density of a powder form of SM is the ratio of the mass of an untapped powder sample and its volume, including the contribution of the inter particulate void volume. However, the bulk density mainly depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per milliliter (g/mL). It is also expressed in grams per cubic centimeter (g/cm^3). The bulking properties of a powder are directly dependent upon the preparation, treatment, and storage of the sample. The particles can be packed to have a range of bulk densities, and, moreover, the slightest variation of the powder layer may result in a changed bulk density. The mean value of the bulk density of Safūf-i Mu'allif powder was found around 0.73.

CONCLUSION: In the current study, the polyherbal Unani formulation Safūf-i Mu'allif was studied with physiochemical standards using modern analytical tools. In respect of the preparation of the formulation, the step by step standard operative procedure was developed as per Qarabadin –I Majidi (A Classical Unani Pharmacopoeia), NFUM and UPI. Among the authentification of the ingredients of the SM, the ingredients used in the preparation were identified and authenticated properly. Therefore, in view of the above-obtained data, interpretation results and discussion it can be concluded that these developed quality standards of SM may help in quality consistency, safety and efficacy of the formulation for preparation and enhance its therapeutic values for future prospective, which may be used as a standard monograph for identification and quality control and also for further future research work.

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