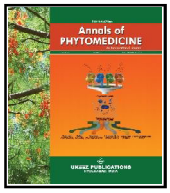
Annals of Phytomedicine 11(2): 814-821, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.2.100

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Standardization and HPTLC fingerprinting of a potent Unani formulation

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Article Info

Article history
Received 30 October 2022
Revised 19 December 2022
Accepted 20 December 2022
Published Online 30 December-2022

Keywords
Quality control
Standardization
Unani formulation (Sharbat-e Zufa Murakkab: SZM)
Hyssopus officinalis L.
HPTLC

Abstract

Unani system of medicine has acquired augmenting apprehensions from clinicians, patients, healthcare systems, and policymakers. Lack of appropriate standardization and characterization of numerous polyherbal formulations, often makes people hesitant to accept it therapeutically. The development of standard operating procedures and physicochemical standardization is very much necessary to maintain batch-to-batch efficacy of the formulations. The safety and efficacy of the formulations depend on the identity, purity and strength of the ingredients. Unani formulation (Sharbat-e Zufa Murakkab : SZM) is based on well-known pharmacopoeial formulation mentioned in a classical Unani book (Bayad-i-Kabir) that is used more frequently as an expectorant to remove the phlegmatic cough and primarily to treat asthma. The present work was intended to establish the various organoleptic and physicochemical parameters including ash values, alcohol-soluble matter, extractive values, pH, reducing and non-reducing sugar, viscosity, specific gravity, refractive index, and high-performance thin-layer chromatography (HPTLC), for the standardization of SZM. The findings of the present study unveiled that SZM is safe to be used for remedial purposes and its batch-to-batch identification is possible for quality control and it could help in preparing consistent and efficacious formulations to be used at mass level.

1. Introduction

Unani system of medicine, an important part of Ayush, is contributing as a traditional system of medicine especially in India and the sub-continent (Husain, 2022). In recent post-COVID years, there has been a resurgence of interest in the indigenous/traditional systems of medicine in developing and advanced countries and a larger population relies on the time-tested traditional/alternative medicines. The medicines obtained from the plant sources are often more effective and safe against several diseases as compared to synthetic drugs (Husain, 2021; Kamboj, 2000).

The therapeutic option of the Unani system of medicine, pharmacotherapy, involves the use of natural origin drugs, most of which are herbal, whereas others are animal- or mineral based having minimum side effects (Husain, 2022; Parveen *et al*., 2020). The basic treatment approach of Unani system is holistic and the pharmacological modalities are based on the natural products from plants or its part. The single drugs or their combinations are used in the case of complex and chronic disorders (Husain, 2021).

The absence of proper standardization and characterization of many polyherbal formulations, their indefinably identified pharmaco-kinetics and pharmacodynamics, and the non-availability of data on the drug-drug and food-drug interactions often make people unwilling in adopting the Unani medicine.

The World Health Organization (WHO, 2011) has emphasized the need to ensure quality control and standardization of medicinal plant products and defined the standardization and quality control of herbal drugs as “the process involved in the physicochemical evaluation of crude drug covering aspects such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, and documentation of safety and risks based on experience”. WHO (2013) also suggested to use the sophisticated modern analytical techniques, which involve several steps; specifically the accurate identity of the drug sample, organoleptic, pharmacognostical and phytochemical assessment, test for biologically active substances, determination of microbial load, heavy metals and aflatoxins (Parveen *et al*., 2020; Rai *et al*., 2020; Alam *et al*., 2019; Folashade *et al*., 2012).

The Unani medications are used either singly or multi-ingredient compound formulations. The formulations consist of various dosage forms such as pills (Goli), tablets (Qurs), powder (Safûf), liquid dosage forms; syrup (Sharbat), distillate (Arq), decoctions (Joshanda) and semisolid dosage forms; Ma’jûn, Khamîra, Itrîfal, La’ûq (NFUM, 2008). The safety, efficacy and purity of compound Unani medicines are mainly depend on the quality, which directly reflects the pharmacological properties of the medicinal product (Husain, 2022). Nevertheless, to establish any standard, only organoleptic parameters (colour, odour and taste) will not be enough to establish the quality standard of the formulation but it requires thorough study based on

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modern analytical techniques to generate evidence based scientific data.

Unani plant-origin based formulations have been proven clinically for their antiasthmatic effect along with biological benefits. SZM is a polyherbal Unani formulation that is prescribed by renowned Unani physicians of the times in the condition of asthma (Diq al-Nafas) and productive cough (Su'âl Ratab) which is also supported by clinical studies (Kabeeruddin, 2008; Sehar *et al*., 2015). The flower of *Hyssopus officinalis* L. (Gul-e-Züfa) is the main ingredient of SZM formulation. In Unani system of medicine, *H. officinalis* is used as expectorant, antiseptic, deobstruent, anti-inflammatory, carminative and vermicidal activity and for the treatment of chronic cough, cold, stomachache, sciatica and colic pain (Khan, 2013). The development of standard operating procedures and standardization are of great importance to ensure the quality and authenticity of the herbal drug. Till now, there are only few reports on its standardization on the basis of limited parameters and without HPTLC (Khan *et al*., 2018). Therefore, the present work has been undertaken for detailed study and with an objective to establish a pharmacopoeial reference standard for SZM on the basis of its organoleptic characteristics and physiochemical standards including HPTLC fingerprinting profile.

2. Materials and Methods

2.1 Procurement and identification of raw drugs

All plant materials of SZM were procured from registered herbalist and put through taxonomical identification and authentication by a botanist of National Research Institute of Unani Medicine for Skin Disorders (NRIUMSD), Hyderabad. The identified drugs were deposited in the museum with voucher specimen numbers as; *Ficus carica* L. (SMPU/CRI-Hyd 13584), *Althaea officinalis* L. (SMPU/CRI-Hyd 13585), *Glycyrrhiza glabra* L. (SMPU/CRI-Hyd 13586), *Iris ensata* L. (SMPU/CRI-Hyd 1387), *Foeniculum vulgare* Mill. (SMPU/CRIHyd13588), *Apium graveolens* L. (SMPU/CRI-Hyd 13589), *Adiantum cappilus-veneris* L. (SMPU/CRI-Hyd 13590), *Hyssopus officinalis* L. (SMPU/CRI-Hyd 13591), *Vitis vinifera* L. (SMPU/CRI-Hyd 13592), respectively.

2.2 Composition of Unani formulation

The composition of Unani formulation (SZM) described (Kabiruddin, 2008) in a Unani classical book (Bayad-i-Kabir) is as follows (Table 1).

Table 1: Composition of Unani formulation: SZM

S. No.	Unani name of ingredients	Botanical name	Part used	Quantity
1.	*Anjîr*	*Ficus carica* L.	Fruit	10 No.
2.	*Tukhm-i Khatmî*	*Althaea officinalis* L.	Seed	10 g
3.	*Asl- al-Sûs*	*Glycyrrhiza glabra* L.	Root	10 g
4.	*Îrsa*	*Iris ensata* L.	Root	10 g
5.	*Bâdiyân*	*Foeniculum vulgare* Mill.	Fruit	15 g
6.	*Tukhm-i Karafs*	*Apium graveolens* L.	Seed	15 g
7.	*Parsiyâoshan*	*Adiantum cappilus-verenis* L.	Whole plant	20 g
8.	*Zûfa Khushk*	*Hyssopus officinalis* L.	Whole plant	20 g
9.	*Mawîz Munaqqa*	*Vitis vinifera* L.	Seedless Fruit	90 g

2.3 Preparation of Unani formulation: SZM

The study Unani formulation was prepared following the method described in a Unani classical book (Bayad-i-Kabir). All the ingredients were weighed and soaked in water overnight. *F. carica* (Anjîr) and *V. vinifera* (Mawîz Munaqqa) soaked separately in a stainless steel pot, followed by kneading with hand and mixed with all other ingredients. The next morning, all the mixed ingredients were boiled on a gas stove, till the pulp softens and the volume of water reduced to half, to get a decoction (Joshanda). Afterwards, the decoction was filtered with a muslin cloth and then kept in a stainless steel pot. Finally, sugar in the ratio of 1:3, was added to the decoction to obtain 'One Tar Qiwâm', and preparation was kept for cooling up to 48 h at room temperature. Afterwards, citric acid and sodium benzoic acid was added to the solution and heated again with continuous stirring to make the Qiwâm (the basic solution of particular required consistency that is generally made over low fire by adding water, distillate or fruit juice with any of the bases of purified honey or sugar) of Sharbat. The obtained formulation was stored in a moisture-free, airtight glass container. The same preparatory measures were adopted for all three batches of the study formulation (Kabiruddin, 2008; NFUM, 2008; Sehar *et al*., 2015).

2.4 Precautions

The solution of particular required consistency (Qiwâm) was tested with a classical parameter and with hand refractometer. The external temperature was regularly monitored to check the viscosity and crystallization of sugar. Stainless steel pots were used for the preparation due to their high corrosion resistance. Continuous stirring was done throughout the process. Water was not added in any form after the formation of Qiwâm, as it could increase the chance of contamination and could spoil the preparation. The preparation was put through a cooling process immediately and moved to the airtight closed glass container and stored in dark places (Kabiruddin, 2008; NFUM, 2008; Sehar *et al*., 2015).

2.5 Assessment of Unani formulation

All the three batches of SZM were analysed by the classical identification procedure as follows:

2.5.1 Classical method 1

A drop of Qiwâm was first placed in between the thumb and finger and then pressed, subsequently; both finger and thumb were separated gradually from each other. During separation, a thin wire-like structure was perceived that was a first degree or Ek Târ Qiwâm or Sharbat (NFUM, 2008).

2.5.2 Classical method 2

To confirm the appropriateness of Qiwâm, one drop of Qiwâm was taken and placed at a solid place. The drop remained round and was not dispersed which indicated that Qiwâm was fully-fledged (Pukhta) and have not required reheating (NFUM, 2008).

2.6 Modern technique to assess the Qiwâm of SZM

Qiwâm of SZM was estimated by the refractometer and readings were recorded. Further modern parameters of standardization including refractive index, viscosity, and specific gravity were also used to assess the Qiwâm.

2.7 Organoleptic properties

Organoleptic properties of SZM including colour, odour, and taste were observed and recorded.

2.8 Physicochemical appraisal of SZM

The prepared SZM was evaluated by total and acid insoluble ash, water, and alcohol soluble matter, extractive value, pH as such, and pH of 50% solution, sugar quantity, viscosity, specific gravity at 25°C, refractive index, and HPTLC fingerprinting (Alam *et al*., 2019).

2.8.1 Ash values

2.8.1.1 Determination of total ash

5 g of SZM was incinerated in a tarred silica crucible by a maximum 450°C temperature until free carbon cooled, and weighed. The total ash percentage concerning the amount of drug was then calculated. The same series of steps were repeated three times with calculating mean and standard deviation.

2.8.1.2 Determination of acid-insoluble ash

The ash was heated with 15 ml dilute HCl in a tarred silica crucible for 5 min. Further, the insoluble stuff was placed on Whatman ash less filter paper and after washing with hot water, ignited by a maximum 450°C temperature for an hour. Finally, it was weighed and the total ash percentage was then calculated concerning the quantity of drug taken. The same series of steps were repeated three times with calculating mean and standard deviation.

2.8.1.3 Determination of alcohol-soluble matter

5 g of SZM was placed in a conical flask and 100 ml of alcohol was also added to the specified mark. The flask was then shaken with the help of a shaker; frequently for 6 h and then stand still for 18 h. Thereafter, it was filtered rapidly; of which, 25 ml of the filtrate was evaporated to dryness in an evaporating dish. Further, it was dried at 105°C, weighed, and calculated the alcohol extractive percentage concerning the quantity of SZM.

2.8.2 Determination of extractive values

The extractive value of SZM in water was calculated by the hot extraction method; 5 g accurately weighed SZM was placed in a conical flask in which 100 ml of water was added and weighed to obtain the total weight including the flask. Afterward, the flask was coupled with a reflux condenser and heated gently for 6 h on the heating mantle. The extracts were then filtered with filter paper and moved to a previously weighed petri dish and evaporated for complete drying on a water bath. Further, the extract was dried in the oven till the weight become constant. After complete drying, the extractive value was determined concerning the weight of SZM.

2.8.3 Determination of pH

The pH of SZM was ascertained by a digital pH meter calibrated by distilled water and buffer.

2.8.4 Determination of reducing and non-reducing sugar

2 g of SZM was dissolved in 250 ml distilled water, followed by adding sufficient amount of saturated lead acetate solution to yield a flocculent precipitate that was shaken thoroughly and stand still for 15 min. Thereafter, the solution was filtered through a dry filter paper. Further sufficient amount of anhydrous sodium carbonate or potassium oxalate was added to make sure that all the lead had been removed then again it was filtered.

2.8.4.1 Reducing sugar

The sample solution prepared earlier was taken into a burette and titrated as above. The titration was completed within a total boiling time of 3-4 min. The sum of reducing sugar was calculated by dextrose used in the above titration. The reducing sugar percentage was calculated concerning the quantity of drug taken. The same series of steps were repeated three times with calculating mean and standard deviation.

2.8.4.2 Non-reducing sugar

50 ml of prepared sample solution of SZM was taken separately into a flask in which 15 ml of HCl was added and heated for 3-4 min. The solution was cooled down rapidly and neutralized by NaOH solution adding phenolphthalein as an indicator and the volume was made up to 250 ml. Further, the non-reducing sugar percentage was calculated concerning the quantity of drug taken. The same series of steps were repeated three times with calculating mean and standard deviation.

2.8.5 Viscosity

The viscosity of all batches was gauged by a 'U' tube viscometer. The SZM fluid was passed through the viscometer by sucking or blowing to the specified weight and the time taken for the fluid meniscus to travel through the two specified marks was measured.

The kinematic viscosity in poise is calculated from the following equation:

$$\text{Kinematic Viscosity} = kt$$

where, k = the constant of the viscometer tube determined by observation on the liquid of known kinematic viscosity, t = time in second for the meniscus to pass through two specified marks (Rai, 2015; Kumar and Prasan, 2013ab).

2.8.5.1 The viscosity of 50% (v/v) aqueous solution of SZM

50 ml SZM was taken accurately in a volumetric flask of 100 ml and made up the volume up to 100 ml by adding the distilled water. The solution was kept on the stirrer until the drug dissolved completely. Sample of SZM was filled up to the half bulb of viscometer. Then a sample of SZM was sucked from the other end of the viscometer above the 'A' mark. The time of flow of sample from point 'A' to 'B', was noted using a stopwatch. The series of steps were repeated with distilled water also and the kinematic viscosity of SZM was then calculated.

2.8.6 Specific gravity

The specific gravity of all batches of the formulation was measured using a pycnometer. A thoroughly cleaned and dry pycnometer was

selected, weighed, and filled with distilled water up to the mark at the neck of the bottle. The exact weight was noted using a weighing balance after maintaining a 25℃ temperature. Then after drying the same bottle was filled with SZM up to the same level maintaining 25°C temperature and weight was recorded. The weight of SZM and water was determined by subtracting the weight of the empty bottle. The specific gravity was calculated by dividing the weight of the SZM syrup and water contained in the pycnometer (Thangarathinam *et al*., 2013).

2.8.7 Refractive index

A drop of syrup (Sharbat) was placed on the refractometer, the cover was then closed, and read the scale (the line at the top of the dark area) quickly. Reading was taken to the nearest 0.1%. The refractometer was dried with tissue paper and rinsed with water after each reading (Thangarathinam *et al*., 2013).

2.8.8 High performance thin layer chromatography (HPTLC)

Qualitative densitometric HPTLC fingerprinting was put into effect to evaluate the SZM. The chloroform extract of SZM was used for TLC application. The analysis was executed on 10 cm × 10 cm on pre-coated aluminium sheets 60 F254 (Merck KgaA, Germany) plates using an automated TLC applicator system of the DESAGA Sarstedt-Gruppe. Many solvent systems with varying volume ratios were tried and a suitable solvent system was selected and developed in the twin trough chamber of TLC to the 80 mm height of the plate to separate the components on the polar phase of silica gel and that of the mobile phase of the solvent system. Band length, distance between the bands, and bottom of the plate were 10 mm, and 10 mm, respectively. The plate was developed only after saturation of the twin trough chamber at room temperature for 20 min. For the mobile phase, toluene, ethyl acetate, and methanol combination were used as a solvent system in the ratio of 7:2:1 respectively. After development, the TLC plate was air-dried and detected for spots at 366 nm and 254 nm with a UV Cabinet system. Further, it was scanned with the Densitometer CD60 of DESAGA Sarstedt-Gruppe system under the UV range of 366 nm, 254 nm. SZM samples were also evaluated for the microbial load, aflatoxins, and heavy metal (Grover *et al*., 2014; Shahnawaz 2020; Deare *et al*., 2014).

3. Results

The SZM was prepared as per the method described in a Unani classical book (Bayad-i-Kabir), as per the ingredients shown in Table 1**.** The organoleptic properties of SZM in all three batches were recorded as moderately viscous, dark brown in colour, pleasant odour and sweet in taste. The total ash values in three batches ranges from 0.61 to 0.84 (%w/w) and acid insoluble ash ranges from 0.03 to 0.04 (%w/w), respectively. The alcohol-soluble matter in three batches of SZM was found in the range of 13.76 to 16.76 (%w/w). The pH values as such of aqueous solution SZM was in the ranges between 4.26 to 4.27 while the pH values of 50% aqueous solution of SZM ranges between 4.28 to 4.29 (Table 2). The reducing sugar of aqueous solution SZM in three batches was found in the range of 25.37 to 27.09 (%w/w) while non-reducing sugar of aqueous solution SZM was in the range 31.61 to 31.97 (%w/w). The viscosity was found to be in the range of 1.00 to 1.03 in three different batches. The specific gravity of SZM was in the range of 1.50 to 1.51 in the three batches which prescribed the identity and purity of the preparation at certain temperatures and pressure. The refractive index of SZM was found to be 1.49 in the three different batches (Table 2).

Table 2: Physicochemical evaluation of Unani formulation: SZM

S. No.	Parameters	Mean ± SD(n=3 readings in each of three different batches)
1.	Total ash (% w/w)	0.61 ± 0.11 to 0.84 ± 0.01
2.	Acid insoluble ash (% w/w)	0.03 ± 0.03 to 0.04 ± 0.05
3.	Alcohol soluble matter (% w/w)	13.76 ± 0.19 to 16.76 ± 1.12
4.	Reducing sugar (% w/w)	25.37 ± 0.95 to 27.09 ± 0.40
5.	Non- reducing sugar (% w/w)	31.61 ± 0.72 to 31.97 ± 1.15
6.	pH as such	4.26 to 4.27
7.	pH (50%) aqueous solution	4.28 to4.29
8.	Viscosity	1.00 to 1.03
9.	Specific gravity	1.50 to 1.51
10.	Refractive index	1.49 ± 0.01

In HPTLC analysis, the chloroform extract spotted on silica gel “G” plate and developed with toluene-ethyl acetate-methanol (7:2:1) as mobile phase the densitogram shows six peaks under UV 366 nm at R_f values 0.01, 0.14, 0.45, 0.54, 0.67, 0.98 and under UV 254 nm in the densitogram showed six peaks at R_f values 0.02, 0.07, 0.19, 0.43, 0.91, 0.98 as shown in Table 3 and Figures 1, 3.

Table 3: Peak lists of chloroform extract of Unani formulation: SZM at UV 366 nm and UV 254 nm

Peak list of chloroform extract of SZM at UV 366 nm					
Peak No.	**Y-Pos**	**Area**	**Area %**	**Height**	**Rf value**
1.	10.3	361.84	45.73	190.88	0.01
2.	19.7	165.90	20.97	50.79	0.14
3.	41.4	134.28	16.97	46.14	0.45
4.	47.5	4.53	0.57	3.26	0.54
5.	56.4	18.12	2.29	6.66	0.67
6.	78.4	106.53	13.46	21.07	0.98

Peak list of chloroform extract of SZM a UV 254 nm					
Peak No.	**Y-Pos**	**Area**	**Area %**	**Height**	**Rf value**
1	10.3	341.99	6.97	238.07	0.02
2	14.1	144.96	2.95	89.45	0.07
3	22.6	30.00	0.61	29.53	0.19
4	39.9	3184.97	64.91	731.43	0.43
5	73.4	621.46	12.67	113.59	0.91
6	78.9	583.37	11.89	198.48	0.98

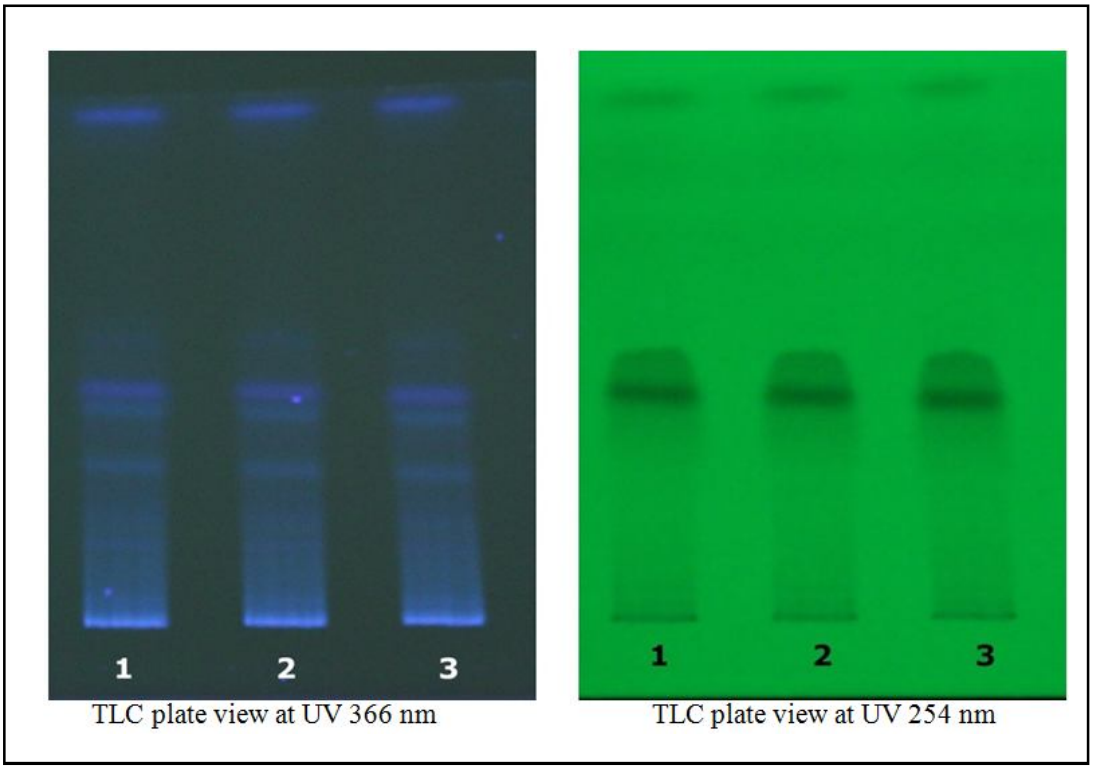

Figure 1: TLC of chloroform extract of Unani formulation: SZM.

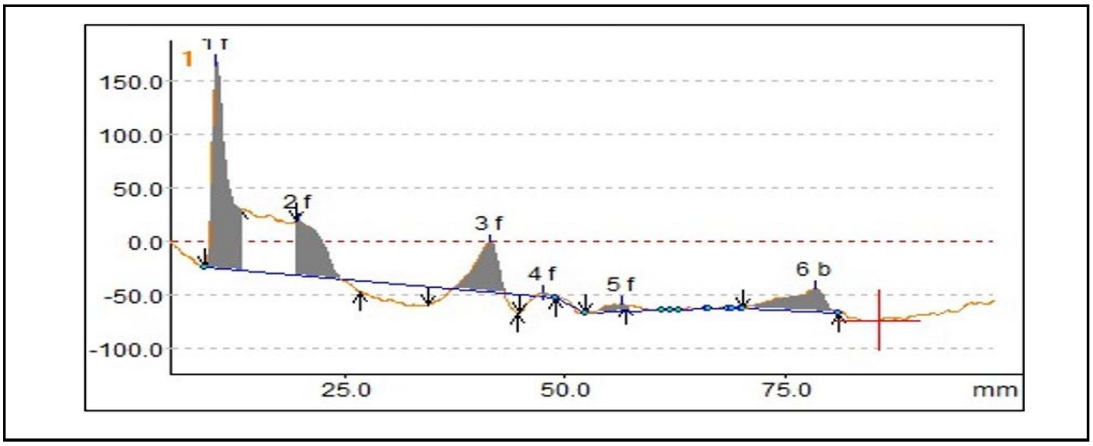

Figure 2: Densitogram of chloroform extract of Unani formulation: SZM at UV 366 nm.

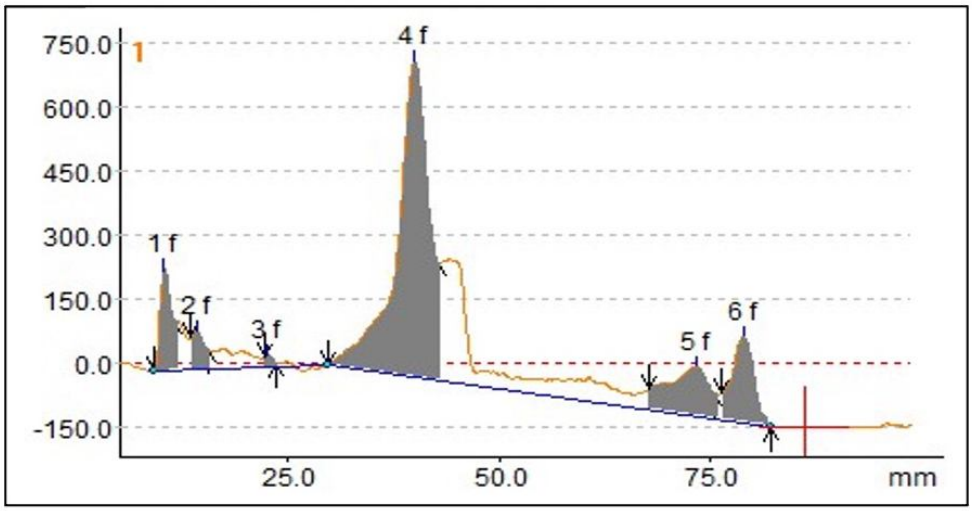

Figure 3: Densitogram of chloroform extract of Unani formulation: SZM at UV 254 nm.

The total microbial plate count (TPC) varies from 60-88 × 10^3, which are found within the permissible limits as per UPI (Anonymous, 2010) and WHO guidelines, additionally, *Salmonella* spp., *Escherichia coli*, and total yeast and mould count were absent (Table 4).

The test report reveals that heavy metals like lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) are found to be nil or below the permissible limit of WHO (Anonymous, 2010; WHO, 2002), therefore it is safe for use (Table 5).

Table 4: Microbial load and aflatoxins contamination

S. No.	Parameter analyzed	Results			Permissible limits as per WHO
		Sample-I	Sample-II	Sample-III	
	Microbial load contamination				
1.	Total bacterial load	60 X 10^3	88 X 10^3	78 X 10^3	Not more than 10^5/g
2.	*Salmonella* spp.	Nil	Nil	Nil	Nil
3.	*Escherichia coli*	Nil	Nil	Nil	Nil
4.	Total fungal count	Nil	Nil	Nil	Not more than 10^3/g
	Aflatoxins contamination				
1.	B1	Nil	Nil	Nil	Not more than 0.50 ppm
2.	B2	Nil	Nil	Nil	Not more than 0.10 ppm
3.	G1	Nil	Nil	Nil	Not more than 0.50 ppm
4.	G2	Nil	Nil	Nil	Not more than 0.10 ppm

Table 5: Heavy metals analysis in Unani formulation: SZM

S. No.	Parameters analyzed	Results	Permissible limits (WHO)
1	Lead-(Pb)	Absent	10 ppm
2	Cadmium-(Cd)	Absent	0.3 ppm
3	Arsenic-(As)	Absent	3.0 ppm
4	Mercury-(Hg)	Absent	1.0 ppm

4. Discussion

The organoleptic properties of SZM were evaluated, the appearance of SZM was moderately viscous inferring that the consistency (Qiwâm) of syrup (Sharbat) was rightly prepared and other drug ingredients were added appropriately. The colours of all three batches of SZM were found to be dark brown as desired. The odour of SZM was found to be pleasant in all three batches. Taste, which plays an important role in herbal products from the consumer's point of view, was found to be sweet in all three batches of SZM.

The physicochemical evaluation was performed on widely accepted parameters to value the purity of drugs which includes ash value, water-soluble extract, alcohol soluble extract, pH value, loss of weight on drying at 105°C, and extractive values (Table 2).The ash value of a drug usually denotes the amount of inorganic matter present in it.

The total ash is the estimate of the overall quantity of material remains after incineration including; physiological ash (plant tissue) and non-physiological ash (metallic or earthy material). The acid-insoluble ash denotes the amount of silicaceous material present in the formulation. If the ash value is higher than standard, it shows the adulteration, contamination, substitution, or negligence in formulation preparation. Generally, the solubility of the drugs is specific and fixed. So, it is an important parameter for establishing the standard of any drug (Rasheed *et al.* 2017).

There are associations between pH and microbial contamination; neutral or alkaline pH favours the growth of microbes in herbal preparations (Abba *et al*., 2008). The overall range of pH as such and 50% aqueous solution of SZM was 4.26-4.28, respectively, which was slightly acidic, therefore the probability of contamination is less (Table 2). Estimation of reducing and non-reducing sugar is important in setting up the standards because many preparations of the Unani system of medicine, either polyherbal or herbo-mineral, are prepared in the honey or sugar base Qiwâm. Viscosity is a property of liquid related to resistance to flow and it is inversely proportional to temperature and pressure which is specific and fixed. It is used as a standard for liquid preparation. The higher viscosity of the formulation indicates a higher amount of adulterants. Specific gravity is the ratio of the specific weight of the material to the specific weight of the distilled water. It varies with temperature and pressure so the sample must be compared with pressure and temperature. It is commonly used as a sample means for obtaining information about the concentration of the solution of various materials such as; sugar, honey, and juice. The refractive index specifies the bending of light or refraction after entering a material that is fixed for the solution, therefore used to confirm the purity of the preparation (Zafar *et al*., 2021).

HPTLC analysis for chloroform extract was quite promising because the extractive yield is maximum. HPTLC technique is one of the most valuable techniques to detect the number of components in the extract and can provide quantitative aspects and peaks recorded in the densitogram. It is an important analytic tool used for identifying adulteration to evaluate the quality of drugs. If the drug is adulterated, secondary metabolites may be present there, which illustrates an increased number of spots (Ansari *et al*., 2020).

Microbes generally appear in the formulation due to the improper drying process of the ingredients of the formulations. Further, microbes decompose improperly processed drugs by multiplying within them and it is hard to stop when started. The alarming signs of microbial attack are off-odour and taste. Also, they could cause some serious infection to the patient if pathogenic bacteria are involved (Ansari *et al*., 2020; Zafar *et al*.; 2021; Rasheed, 2017).The natural ingredients are rarely devoid of microbial contamination, thus, quality assurance is required to check the microbial presence quantitatively or qualitatively which should be within the permissible limit set by WHO (2002). However, potentially harmful microbes (*e.g*., *E. coli* and *Salmonella* spp.) and aflatoxins should never be present in a formulation. The detection of aflatoxins B1, B2, and G1, G2 was found to be within the permissible limit (Table 4).

The heavy metals usually stored in the plant through the soil are toxic. Ingestion of such contaminated plant products may lead to various health issues and diseases like high blood pressure, change in heart rhythm or paralysis, toxicity, and even death. Hence, it is recommended by WHO that every herbal product or mineral-based drug, therefore, should be analyzed for heavy metals (Zafar *et al*.; 2021; Rasheed, 2017).

5. Conclusion

The establishment of Standard Operating Procedures (SOPs) is necessary to ensure the quality product and its uniform availability everywhere. In this study, the frequently used Unani formulation (Sharbat-e Zufa Murakkab: SZM) was put through the development of standard operating procedures instituting its authenticity, identity, quality, purity as well as detection of adulterants. Based on the organoleptic and physicochemical analysis, certain standards had been prescribed along with other parameters such as; heavy metals analysis, aflatoxins contamination, and high performance thin-layer chromatography (HPTLC). Based on these findings the formulation was brought up in determining and ascertaining its quality and standardization. Thus, the study is beneficial in quality assurance including safety and efficacy of the Unani formulation: SZM, moreover, other scientific evaluations can be done accordingly in near future.

Acknowledgements

The authors are extremely thankful to the Director General, Central Council for Research in Unani Medicine (CCRUM) for providing necessary research facilities.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Mohd Uzair Beg, Mohammed Abdul Rasheed Naikodi, Mohd Kashif Husain, Munawwar Husain Kazmi and Javed Inam Siddiqui (2022). Standardization and HPTLC fingerprinting of a potent Unani formulation. Ann. Phytomed., 11(2):814-821. http://dx.doi.org/10.54085/ap.2022.11.2.100.**