Original Article

Pharmaceutico-analytical Study of *Kushtae Shangarf* Prepared with *Jozbua* (*Myristica fragrans* Houtt.) and *Phitkari* (Alum)

Mohd Akhtar Ali, Hamiduddin, Mohammad Zaigham, Mohammad Ikram, Rajeev Ranjan

Department of Ilmul Saidla (Unani Pharmacy), National Institute of Unani Medicine (NIUM), 1Department of Materials Engineering, Indian Institute of Science, Bengaluru, Karnataka, India

**Introduction:** *Kushta* of *Shangarf* (KS) is in therapeutic use since long time in *Unani* and Ayurvedic medicine. It requires extensive assessment with respect to its safety and processing because of the presence of mercury. KS prepared with *Shangarf* (*HgS*), *Phitkari* (alum), and *Jozbua* (*Myristica fragrans* Houtt.) was selected for the study. It is indicated as tonic, increases blood production, and improves complexion of face. Preparation of KS by classical and contemporary method and its comparative physicochemical analysis was attempted in this work. **Materials and Methods:** *Kushta* was prepared by incinerating the drugs kept inside *Buta* in *Bhatti* with 24 kg of *Uple* (cow-dung cakes) and also in muffle furnace. Samples obtained were evaluated by *Unani* specifications (test), powder characterizations, loss on drying, pH, ash value, solubility, particle size, and qualitative estimation of organic and inorganic constituents, X-ray diffraction (XRD), quantitative estimation by inductively coupled plasma–mass spectrometry and inductively coupled plasma–optical emission spectrometry, and so on. **Results:** Physicochemical standards set in were comparable in KS prepared by classical method (KSCM) and in KS prepared by muffle furnace method (KSMF), except water-soluble ash and solubility in water were found slightly more in KSMF. XRD study revealed the presence of aluminum oxide phase and absence of mercury in both the samples. Quantitative estimation of elements in both the samples in decreasing order are as follows: sulfur > aluminum > calcium > iron > arsenic. Arsenic was found more than iron in KSCM at parts per million level. **Conclusion:** Preliminary understanding suggests that muffle furnace method could be a better option with respect to safety and ease of preparation. *Shangarf* incinerated above 900°C with *Phitkari* and *Jaiphal* did not show presence of mercury in both the samples, indicating KS prepared by incinerating at higher temperature can be safer than unroasted preparation. Studied formulation can be recommended or used for its indications without the concern of mercury toxicity.

**Keywords:** Ayurvedic, cinnabar, Kushtae Shangarf, mercury, toxicity, Unani medicine

**Address for correspondence:** Dr. Hamiduddin, Department of Ilmul Saidla (Unani Pharmacy), National Institute of Unani Medicine (NIUM), Kottigepalya, Magadi Main Road, Bengaluru, Karnataka 560091, India. E-mail: drhamid2003@rediffmail.com

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converted either into their carbonates or oxides by the process of Ibraq (roasting) or Taklees (calcination), so as to make them compatible, the product obtained is known as Mukallas or Kushta (calcined).\[1\] Kushtan in Persian means “to kill.” Kushtas are made to make the drug easy to use and purposeful, to remove the harmful and disadvantageous materials from it, to minimize the dose and increase the potency of the drug,\[1\] and to use in particular disorders that are not curable by pure herbals alone. The general procedures for synthesis of Kushta begin with the process of purification (Ghast-e-Adviyah), cleaning (Tasfiyah), and detoxification (Tadbire-Adviyah).\[2,3\] The efficacy of Kushta depends on the method of preparation and other drugs used during its preparation, for example, in one method, the action of a particular Kushta is Mushil (purgative) and on changing the method, it can display opposite action such as Qabiz (constipative).\[1,4\] The most ancient manuscript written on the field of Kushtajat by Baracoles is “Asagharba,” which means collection of varieties. This terminology was used for drugs of mineral origin used in the treatment of diseases and its transformation in various dosage forms.\[1\] Some metals have the potential to produce adverse effects, thus during alteration of the metals in the form of Kushta, it is essential to evaluate the margin of safety between the dose level that produces the therapeutic effects and adverse effects, that is, to provide benefit-to-risk assessment.\[5\] It can be achieved by analytical procedures and animal experiments.

In this work, Kushta of one such metal ore, that is, Shangarf (cinnabar) was evaluated. Kushta of Shangarf is in therapeutic use since long but now it requires extensive assessment in view of the presence of mercury in sulfide form. Shangarf (cinnabar) has been used since 2000 years in traditional medicine. Shangarf/cinnabar (HgS) is red sulfide of mercury. It is found as a very heavy mineral ore of mercury and also artificially prepared by heating mercury with sulfur. Shangarf is generally used internally in the form of Kushta (incinerated). It is also used in Unani and Ayurvedic medicine in different forms. Actions of Kushtae Shangarf (KS) documented in Unani medicine are Muqawwie bah (aphrodisiac), Muwallid-i-Mani (Increase semen production/ spermatogenic), Muqawwiea’ saab (nerve strengthening), and so on. It is used in brain and nerve diseases, sexual debility, Balghami (phlegmatic) and cold diseases, Falij (paralysis), Tashamnej (spasm), tetanus, palsy, tremors, general body weakness, to increase physical strength of a man, anorexia, Aateshak (syphilis), Dama (asthma), Zeequin nafas (bronchial spasm), Sua’le muzmin (chronic bronchitis), arthritis, chronic and compound fever, to improve skin color and increase glow of face, and so on.\[3,6,7\] All these indications reveal its use in some grave ailments also. Few studies are conducted on cinnabar and its Bhasmal Kushta in experimental animal (rats), which reveal that Marita Hingula (processed and incinerated cinnabar) is safer than that of Shoddita (processed).\[8\] Shuddha Hingula (pure processed) proved to be nontoxic, safe, and effective in acute and subacute toxicity studies.\[9\] Besides this, various other physicochemical parameters are needed to be evaluated in KS with the help of sophisticated instruments. There is also a need of comparative evaluation of classical as well as conventional method of KS preparation for appropriate comment on safety issues. In this study, formulation of KS prepared with Shangarf, Phitkari (alum), and Jozbua (Myristica fragrans Houtt.) with dosage of one Chawal (15 mg) was selected as mentioned in Kitab al-Taklees by Mohammad Kabiruddin. Besides regular uses of general KS, this Kushta is particularly indicated as tonic for Azae raesa (vital organs), increases blood production, and improves complexion of face.\[1,3\] This formulation has been selected because of its ingredients and indications. This particular study is the need of the hour to further uncover the scientific facts regarding safety of this particular incinerated (KS) formulations and similar preparations used in Unani and Ayurvedic medicine. Objectives of the study were preparation of KS as per classical and contemporary method and its comparative physicochemical analysis for its safety assessment.

**Materials and Methods**

Study was carried out in the Department of Ilmul Saidla, National Institute of Unani Medicine (NIUM), the Indian Institute of Science (IISc) Bengaluru, Karnataka, India, and other accredited laboratories. Shangarf, Phitkari, and Jozbua (Myristica fragrans Houtt.) were procured from apothecary shop in Bengaluru. Lemu (lemon) was purchased from the local market of Bengaluru. Samples of drugs in the formulations were submitted at the NIUM Museum with voucher specimen no.: 39/IS/Res/2016.

Shangarf and Phitkari were identified by Regional Ore Dressing Laboratory, Bengaluru, the Indian Bureau of Mines (IBM), Ministry of Mines, Government of India (no.: K-26011/5/BNG-ODL, report investigation no.: IBM/BNG/R.I.M.A. no.652). Jozbua was authenticated by the experts at the Institute of Trans-Disciplinary Health Sciences and Technology (FRLHT) Bengaluru (FRLHT Acc. No. 3820).
Method of detoxification of Shangarf

Shangarf was detoxified by Aabe Lemu (lemon juice) as mentioned in classical literature. Lemon juice was first finely powdered in mortar and pestle manually, then its grinding/ Arrayed triturating process was carried out for four Pahar (12 h) with Aabe Lemu in electric mortar and pestle in the pharmacy of NIUM. Shangarf was incorporated in Kushta formulation after Musaffal/Mudabbar process, as per classical reference regarding the use of metal in Kushta formulation.[1,3,4]

Kushta Shangarf preparation

Mudabbar Shangarf paste was made into pellet manually of 12 g each. It was then kept for drying under shade.

Preparation of Kushta Shangarf by classical method

Phitkari (alum) 5 Tola (60 g) and Jozbua (Myristica fragrans Houtt.). 5 Tola (60 g) were taken and powdered separately, then in clay crucible (Buta) a layer of Phitkari powder was first spread, on it Shangarf 1 Tola (12 g) in the form of pellet was kept, and on it powder of Jozbua was spread in a layer, then the crucible was covered with clay lid, and sealed by the process of Gille Hikmat and Kaproti seven times.[1]

Put (incineration/calcination) process

A pit was prepared, which can accommodate 24 kg cow-dung cakes (approximately 2.5 feet in height and 2 feet in width). After drying in the shade, the Buta was placed in a pit and ignited with 25 Ser (24 kg) Uple (cow-dung cakes) at the place secured from wind, then after cooling white drug part was separated and stored for further use/analysis.[1] Changes in drugs subjected to incineration and quenching media were keenly observed, and heat pattern was noted during the incineration process with the help of thermocouple, sensor of which is placed near Buta in the pit [Figure 1].

Preparation of Kushta Shangarf by muffle furnace method

First, Buta prepared with same ingredients and method mentioned above was taken [Figure 2]. The temperature used to develop a thermogram for the gradual increase and decrease of temperature recorded in the classical method of preparation was followed on muffle furnace for the preparation of Kushta, the temperature of 984.33°C was set with a gradual increase from atmospheric temperature (25°C approximate onward), and then it was again set to the same level of atmospheric temperature to allow it to cool [Figure 2]. Quantity of KS prepared was observed.

Physicochemical Analysis

Organoleptic characters: Prepared Kushta was evaluated for its color,[1,10] odor,[10,11] and taste.[10,11]

Unani specification (test): The floating test,[10,11] fineness test,[10,11] loss of metallic luster,[10,11] wall stick test,[10] and smokeless test[3,6,11] parameters were used for the analysis of proper incineration process [Figure 3].

Powder characterizations: Bulk density and tapped density were determined.[12]

Hausner ratio, compressibility index was calculated, the angle of repose was also determined by using fixed funnel method.[13]

Loss of weight on drying at 105°C,[11] determination of pH,[11] determination of total ash,[11] determination of acid insoluble ash,[11] determination of water-soluble ash,[11] and determination of sulfated ash[11] was carried out as per methods mentioned in the protocol for testing of Ayurvedic, Siddha, and Unani medicine.

Solubility: The solubility in petroleum ether,[14] solubility in alcohol,[14] and solubility in water[14] were done as per the Physicochemical Standards of Unani Formulations.

Particle size analysis: By mesh size: (The Kushta was passed through different mesh number: 80, 100, 170, 200, 240, 300, 350, etc., and the percentage (%) of material passed was recorded).

By X-ray diffraction (XRD) method: (Detail of procedure is mentioned in X-ray diffraction test)

Qualitative estimation of constituents using chemical method

Organic: The presence of alkaloids was tested by Dragendorff’s test[14] and Mayer’s test;[14] phenols, resin, glycosides, and steroids by Salkowski reaction; proteins by Millon’s test; carbohydrates by Fehling’s test; and flavonoids and saponins by the method mentioned in standardization of single drugs of Unani medicine.[15]

Terpenoids: A total of 5 mL of aqueous extract of KS was taken in a test tube and 2 mL of chloroform was added, then 3 mL of concentrated sulfuric acid was mixed in solution. If reddish brown interface is formed then it is an indication of the presence of terpenoids.[16]

Tannins: A total of 5 mL of aqueous extract of KS was taken in a test tube and few drops of 1% solution of lead acetate were added. If yellow precipitate is formed then it is an indication of the presence of tannins.[14]

Inorganic: Calcium, aluminum, cadmium, iron, lead, manganese, and zinc compounds were tested as per the method mentioned in Physicochemical Standards of Unani Formulations.[14]
Mercury compounds: (1) Approximately 20–25 mg of ash of the drug was dissolved in 1 mL of distilled water, and 2 M sodium hydroxide was added until solution become strongly alkaline. A dense yellow precipitate formed indicates the presence of mercury compounds. (2) Approximately 20–25 mg of ash of the drug was dissolved in 1 mL of distilled water, and added carefully into potassium iodide
solution. A red precipitate formed that dissolves in an excess of reagent indicates the presence of mercury compound.\textsuperscript{[14]}

** Arsene compounds:** Approximately 20–25 mg of ash of the drug was dissolved in about 2 mL of distilled water and an equal volume of hypophosphorous reagent
was added. A brown precipitate formed indicates the presence of arsenic.[14]

**XRD study:** XRD is a tool used for the investigation of the fine structure of matter, special arrangement of structural units of substance in its crystalline state, phase equilibria and the measurement of particle size, and the orientation of one crystal or the ensemble of orientations in a polycrystalline aggregate. It is a rapid and accurate method for identifying the crystalline structure, and the sample can be easily prepared. Some limitations of XRD are that it does not help in case of amorphous solid and trace element detection is often difficult. It can be applied for the differentiation among various oxides/sulfide of metals.[17,18] It works on the principles of Bragg’s law written in the form: 
\[
\lambda = 2d \sin \theta
\]
where \(\lambda\) is the wavelength of the X-ray used (1.5406 Å), \(d\) is the interplanar spacing, \(\beta\) is the full width at half maximum of the Bragg peak, and \(\theta\) is the Bragg angle of the Bragg peak (peak position). Basic relationship for determination of particle size using XRD pattern is \(0.9 \beta / \lambda \cos \theta\). XRD studies conducted on KS are confirmed by comparing \(d\)-identified values with \(d\)-standard peak values.[17]

**ICP-OES:** Inductively coupled plasma–optical emission spectrometry (ICP-OES) is a powerful and widely used analytical technique used for the determination of traces elements.[19] It is a multielement analysis technique that uses inductively coupled plasma source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light at a characteristic wavelength. A detector measures the intensity of the emitted light and calculates the concentration of that particular element in the sample. Detection limits are in single and sub-parts per billion range.[20] Characteristics of ICP-OES are appreciable degree of ionization for many elements, high stability leading to excellent accuracy and precision, excellent detection limits for most elements (0.1–100 ng mL\(^{-1}\)), and cost-effective analyses.[21]

**ICP-OES analysis by digestion of juices method: Name and specification of the instrument:** ICP-OES instrument of the make: Teledyne Leeman Labs, Hudson, USA; model: Prodigy 7; instrument condition: spectrophotometer temperature: 35°C, axial time: 30 s; and nebulizer: 37 PSi1, Azyme Biosciences Laboratory, Bengaluru, India was used.

**Procedure:** Weigh 10 mg of sample into Teflon PFA digestion, add 10 mL concentrated HNO\(_3\) and 2 mL concentrated H\(_2\)SO\(_4\) to the sample, cap the vessel, digest the sample in the microwave digester, cool for approximately 5 min, vent and open the vessels, add 3 mL ultrapure H\(_2\)O\(_2\) to complete the digestion of remaining sugars. When effervescence ceases, transfer the samples into clean containers, dilute to 100 mL with deionized water, store it in clean polythene bottles, and aspirate the prepared solution into ICP-OES.

**ICP-MS:** Inductively coupled plasma–mass spectrometry (ICP-MS) is a multielemental analytical method. The Robust–Duo can handle majors, minors, and traces in one method. Advantage of ICP-MS is very low limits of detection in the range of parts per million or below for solid sample and in the range of parts per billion or below for solution sample.[22] Detection limits are 10–100 times superior to those
of ICP-OES and better than graphite furnace atomic absorption spectroscopy. Detecting and quantifying 85% of all elements comes down to concentrations not measurable by other techniques, allowing a unique holistic approach. By using ICP-MS, all kinds of materials can be measured as the ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer. Detection limits are usually in the nanogram per liter range and below (highly sensitive and high resolution). Its applications is in quantitative analysis and survey analysis of main and ultra-trace elements in both organic and inorganic samples.

**ICP-MS analysis by ash method**: Sample name: powder sample A and B (ash method), CA no.: 00316037184 and 00316037185, protocol no.: DRUG|MOH|004. Done at Shiva Analytical Bangalore.

**Procedure**: Weigh 0.3 g of sample in crucible and burn the sample in Bunsen burner up to grey ash. Keep in muffle furnace at 550°C ± 50°C for 2–3 h. Add 7–8 mL of aqua regia (1:3, nitric acid:HCl). Boil on hot plate until all the nitrous fumes are removed. Cool and transfer the solution into 50 mL of volumetric flask with milli-Q water and read to aspiration.

**Determination of sulfur**: Preparation of solution is carried out by carbon tetrachloride saturated with bromine barium chloride (10% solution) in water. **Procedure**: Take 0.5–1 g powdered sample in 250-mL beaker. Add 10 mL carbon tetrachloride saturated with bromine. Keep in cold condition in fume chamber overnight. Add 10–15 mL concentrated nitric acid. Digest on water bath. Add 10 mL of concentrated hydrochloric acid and digest it to expel nitrate fumes till syrupy mass. Cool and extract with hydrochloric acid and make volume to 100 mL. Boil and filter through Whatman number 40 filter paper. Wash the residue with hot water. Filter through Whatman number 41 paper in 600-mL beaker. Acidify the filtrate with hydrochloric acid. Add 20 mL of 10% barium chloride solution. Stir the solution and digest on burner. Allow to settle BaSO4 precipitate overnight. Filter the precipitate through Whatman number 42 filter paper. Wash the precipitate with water. Ignite the precipitate in muffle furnace in pre-weighed platinum crucible up to 850°C. Allow to cool and weigh. Each gram weight of precipitate is equivalent to 0.13734 g of sulfur.

**RESULTS**

**Identification**: Raw drugs procured for preparation of formulations/Kusht were identified as Shangarf (cinnabar), Phitkari (alum) from IBM, Bengaluru (no.: K-26011/5/BNG-ODL, report investigation no.: IBM/BNG/R.I.M.A. no.652), and Jaiphal (Myristica fragrans Houtt.) from FRLHT, Bengaluru (FRLHT Acc. no. 3820).

**Mudabbar process of Shangarf**: For 800 g of Shangarf, quantity of Aabe Lemu (lemon juice) used was 1050 mL for the duration of four Pahar (12 h). For each 400 g of Shangarf grinded in one set of electric mortar and pestle, lemon juice used in the first 4 h was 180 mL, for

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*Figure 4: Particle size Samples 1–4*
the second 4 h was 175 mL, and for the third 4 h was 170 mL (total: 525 mL).

Difference was observed between Mudabbar (processed/detoxified) and non-Mudabbar.

Study on Shangarf (unprocessed): The unprocessed Shangarf was vermilion, shining with white streaks in color, odorless, hard in consistency, particle size was found to be 89 nm (XRD method), pH in water 1% and 10% was 6.73 and 6.81, whereas pH in lemon juice in 1% and 10% was 2.75 and 2.85, respectively. It is not soluble in water [Figure 5].

Study on Shangarf Mudabbar (processed): The processed Shangarf was not shining, deep red in color, slightly citrusy in smell, soft like powder in consistency, reduced and finer/less in particle size, that is, 65 nm (XRD method) in comparison to unprocessed one, a slight increase in weight was noted, pH in water 1% and 10% was 6.85 and 6.90, whereas pH in lemon juice in 1% and 10% was 2.96 and 2.99 respectively. It was slightly soluble in water. Completely dried pellets prepared of processed Shangarf displayed a smooth, flat surface [Figure 6].

Temperature pattern: The mean temperature pattern of thermogram in KSCM is shown in Figure 2. Mean of maximum temperature reached is 984.33°C. This temperature pattern of thermogram followed in KSMFM is shown in Figure 2.

The mean quantity of KS obtained by different methods of preparation in sample KSCM and KSMFM was 1.386 g and 2.37 g, respectively, two different colored materials, that is, white and black was found in Buta after incineration in both the samples as mentioned in Unani classical literature for selected formulation.

Physicochemical studies: The following values were found in white part of two in-house prepared samples of KS prepared by KSCM and KSMFM. The data are based on multiple observations.

Organoletic properties
Color: Slight reddish white colored sample prepared by KSCM and whitish colored sample prepared by KSMFM were obtained. White color was dominant in both the samples; after grinding, both the samples show greyish white color.

Odor: Both the samples (KSCM and KSMFM) were found to be odorless.

Taste: Both the samples (KSCM and KSMFM) were found to be tasteless.

Unani specification test: For result refer Table 1 [Figure 3].

Physicochemical parameters
For results of total ash, acid insoluble ash, water-soluble ash, sulfated ash, solubility in ether, solubility in alcohol, solubility in water, loss of weight on drying, pH and powder characterization refer Tables 2 and 3.

Figure 5: X-ray diffraction pattern of Sample 1 (Shangarf powder)
Qualitative estimation of constituent/element

**Organic:** Alkaloids, phenols, resin, flavonoids, saponins, glycosides, terpenoids, tannins, steroids, proteins, and carbohydrates were found to be negative in KS.

**Inorganic:** Qualitative estimation of inorganic constituents is shown in Table 4.

**X-ray diffraction study**

Sample 1 SP (Shangarf powder) and Sample 2 SMP (Mudabbar Shangarf powder): Sample 1 SP and Sample 2 SMP show the presence of cinnabar (HgS) phase. Comparative graph also shows same pattern, whereas particle size in Sample 1 SP was found to be 89 nm and in Sample 2 SMP was 65 nm. XRD method of particle size analysis was performed [Figure 4]. Intensity of the peaks in XRD pattern shows crystallization in Sample 1(SP) better in comparison to Sample 2 (SMP) because the intensity of XRD peaks is slightly high in Sample 1 [Figures 5 and 6].

**Findings in Sample 3 (KSCM) (white part), Sample 3A (KSCM) (black part), Sample 4 (KSMFM) (white part), Sample 4A (KSMFM) (black part), Sample 5 (incinerated cinnabar), Sample 6 (incinerated alum):** Findings of obtained KS part were recommended for use in classical text (i.e., white part), Sample 3 [Figure 7] shows that it contains broad Bragg peaks, the peak positions of which matched with the phase aluminum oxide (Al₂O₃). Sample 4 consists of the broad peaks of Al₂O₃ along with sharp peaks of Al₂O₃ (corundum). Both Samples 3 and 4 [Figure 8] confirm the presence of aluminum in oxide form, no phase for mercury was detected in both the samples [Figures 7–9].

It appears that while heating the alum, the first product to form is the Al₂O₃ phase (Sample 3), which on further heating (at higher temperature) leads to the formation of Al₂O₃ that has a very large particle size compared to Al₂O₃. The particle size analysis carried out for the Al₂O₃ phase (whose Bragg peaks show very large width) showed very fine sizes, that is,
2.80 nm for Sample 3 (KSCM) and 3.62 nm for Sample 4 (KSMFM). Interestingly, the particle size of the coexisting phase Al₂O₃ in Sample 4 is significantly large as evident from the very small width of the Al₂O₃ peaks. Phase found in Samples 3 and 4 are both crystalline with difference in particle size [Figure 4]. No phase of mercury was noted in Sample 3 (KSCM) and Sample 4 (KSMFM). Crystalline phase of sulfur is not found. Sulfur may be present in noncrystalline (amorphous) phase. Sample 3A (KSCM) black part matches in term with Sample 3 (KSCM) white part and Sample 4A (KSMFM) black part matches in term with Sample 4 (KSMFM) white part. Sample 4A shows minor phase of CaS (calcium sulfide) along with major aluminum phase as Al₂O₃. In Sample 5, no phase was detected as this was the sample containing only incinerated cinnabar, and on macroscopic appearance, it was probably elemental mercury volatilized from cinnabar and collected from the outside of clay crucible in other part of muffle furnace. It was not in the crystalline form but it was in liquid form. XRD might have not shown the same because it detects in crystal form.

Sample 6 (incinerated alum) showed the presence of aluminum oxide phase [Figure 9], matching both Samples 3 and 4 of KS. Extra Al₂O₃ phase was present in Sample 4 [Figures 7–10].

**DISCUSSION**

Debate on the use of heavy metal and particularly, any mercurial preparation in any form in traditional

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**Table 3: Powder characterization**

| Parameters in mean ± SEM | KSCM | KSMFM |
|-------------------------|------|-------|
| Bulk density (g/mL)     | 0.0279 ± 0.00 | 0.0283 ± 0.001 |
| Tapped density (g/mL)   | 0.047 ± 0.001 | 0.0552 ± 0.004 |
| Hausner ratio           | 1.676 ± 0.056 | 1.943 ± 0.114 |
| Compressibility index (%) | 40.211 ± 2.079 | 48.177 ± 3.256 |
| Angle of repose (θ)     | 52.266 ± 1.348 | 52.659 ± 2.371 |

SEM = standard error of mean, KSCM = *Kushtae Shangarf* prepared by classical method, KSMFM = *Kushtae Shangarf* prepared by muffle furnace method

**Table 4: Qualitative estimation (inorganic)**

| S. no. | Name of compound/element | KSCM | KSMFM |
|--------|--------------------------|------|-------|
| 1.     | Calcium compounds        | Positive | Positive |
| 2.     | Aluminum compounds       | Positive | Positive |
| 3.     | Arsenic compounds        | Negative | Negative |
| 4.     | Cadmium compounds        | Negative | Negative |
| 5.     | Mercury compounds        | Negative | Negative |
| 6.     | Iron compounds           | Positive | Positive |
| 7.     | Lead compounds           | Negative | Negative |
| 8.     | Manganese compounds      | Negative | Negative |
| 9.     | Zinc compounds           | Negative | Negative |

KSCM = *Kushtae Shangarf* prepared by classical method, KSMFM = *Kushtae Shangarf* prepared by muffle furnace method

**Figure 7:** X-ray diffraction pattern of Sample 3 (*Kushtae Shangarf* prepared by classical method [KSCM], white part)
medicine is very relevant in the present context. It is very essential to evaluate the margin of safety and collect further data that will provide benefit-to-risk assessment regarding KS preparations. A comparative analysis between classical method of preparation (KSCM) and contemporary method of preparation (KSMFM) has been reported in this work.

Preparation of KS: Mean of maximum temperature reached (984.33°C) in the Bhatti was attempted to maintain with the stay duration to simulate the heating atmosphere in muffle furnace. Highest temperature with its stay duration would be maintained properly in muffle furnace because of better regulatory and controlled condition, this might be the reason for

Figure 8: X-ray diffraction pattern of Sample 4 (Kushtae Shangarf prepared by muffle furnace method [KSMFM], white part)

Figure 9: X-ray diffraction pattern of Sample 6 (incinerated alum)
obtaining more quantity of KS when prepared by muffle furnace method. It was observed that the quality of Uple (cow dung) is very important because low quality of Uple do not produce heat of required intensity in the process of incineration, resulting in incomplete/Kham Kushta with less output, even if recommended quantity of Uple is maintained as per the classical text. The good quality of Uple gives high temperature, but it was observed that they also cool fast such that after 3 h, it cools and reaches till 300°C, whereas the MF cooling pattern is very slow, it cools to 300°C in 7–8 h. It was also observed that increasing the quantity of Uple also increases the quantity of Kushta.

Physicochemical analysis: It was observed that the color of Kushta differs slightly by difference in heat pattern, if heat is below 900°C, the color of Kushta is reddish, and at above 900°C, the color is more whitish. Both KSCM and KSMFM samples that passed the Unani specification test show perfectness of Kushta as per the classical norms [Table 1]. Very poor flow property was observed in KSMFM in comparison with KSCM, indicating lesser density and more bulk in it and is suggestive of finer Kushta and less flow property, which are evident by the classical text confirming the quality of Kushta drug by floating test, wall stick test, and so on. Acidic pH obtained in both the Kushta samples, which was found to be 6, can be used as one of the parameters for its quality standards. Higher ash value indicates the presence of more inorganic constituents in the formulations as evident in case of Kushta formulations. The ash values of above 99% may also be indicating toward Kushtae Kamil/complete Kushta. The solubility of the KSCM and KSMFM in ether, alcohol, and water was found to be very less. These findings can give an idea regarding pharmacokinetic of drug, it can also act as an index of purity. Qualitative estimation of constituents/elements shows absence of all organic constituents. Incineration process might have degraded all the organic constituents. Qualitative test for inorganic constituents such as arsenic, cadmium, mercury, lead, manganese, and zinc compounds was found to be negative in KS, whereas calcium, aluminum, and iron compounds were found to be positive in KS [Table 4]. This result is contrary to the finding of ICP, which shows the presence of arsenic in traces/parts per million level [Table 5].

XRD study: Sample 1 (Shangarf powder) and 2 (Mudabbar Shangarf powder) were identified as cinnabar (HgS), particle size in Mudabbar Shangarf powder has been reduced after grinding in lemon juice in processed cinnabar, it shows probably the effect of grinding with lemon juice. In both Samples 3 (KSCM) (white part) and 4 (KSMFM) (white part), oxidation was observed in XRD pattern. Both samples show the presence of Al_{2.66}O_{4} phase, no other phase is detected except additional phase in Sample 4, which looks like Al_{2}O_{3} (corundum) [Figures 7–10]. From the formulae of the two phases, it is evident that in Al_{2.66}O_{4} for each aluminum metal, 1.5038 oxygen are present and in Al_{2}O_{3}, for each aluminum metal, 1.5000 oxygen are
present. Thus \( \text{Al}_2\text{O}_3 \) is slightly rich in oxygen content than \( \text{Al}_2\text{O}_4 \). XRD finding reveals that no mercury phase was detected in percentage level in Samples 3 (KSCM) [Figure 7] and 4 (KSMFM) [Figure 8] after incineration. Crystalline phase of sulfur was also not detected; sulfur was present in noncrystalline (amorphous) phase and in other form of sulfur such as CaS. Presence of sulfur was confirmed by gravimetric method. Particle size analysis by mesh size shows that it passes through the available 350 number mesh (higher mesh number can also be attempted), and particle size calculated by XRD shows 2.80 nm for KSCM and 3.62 nm for KSMFM with Bragg peaks with very large width confirming very fine particle size. Interestingly, the particle size of the coexisting phase \( \text{Al}_2\text{O}_3 \) in Sample 4 is significantly large as evident from the very small width of the \( \text{Al}_2\text{O}_3 \) peaks. This increase in particle size of \( \text{Al}_2\text{O}_3 \) in Sample 4 (KSMFM) or large grain size in comparison with Sample 3 (KSCM) may be due to grain growth [Figure 4].

White part was found placed above black in both the samples of KSCM and KSMFM. In both the samples, black part match with white part; Sample 4A of KSMFM white part shows minor phase of CaS along with major aluminum phase as \( \text{Al}_2\text{O}_3 \). Calcium is found in \textit{Jaiphal} [28] and sulfide in \textit{Shangarf} as per the literature reviewed. Classical text indicates the presence of white and black part, which was confirmed by the work. [3] Classical text also indicates the use of white part as it was considered perfect. Significance of the presence of white and black part needs further sophisticated investigation. The mean quantity of KS observed by different methods of preparation was 1.386 g in KSCM and 2.37 g in KSMFM. This difference may be due to the difference in heat pattern. More quantity of whitish \textit{Kushta} in KSMFM may be due to proper heat exposure and oxidation.

ICP-OES and ICP-MS analysis by both the processes shows the absence of mercury (Hg < 0.1 mg/kg) but shows the presence of iron as Fe and aluminum as Al in milligram per kilogram. Aluminum was present in better quantity than iron (Fe), whereas presence of arsenic (As) was also noted in parts per million level. In ICP-OES analysis, the results were on borderline of permissible limit of As, that is, 3 ppm [Table 5]. The percentage of sulfur in Sample 1 (KSCM) and Sample 2 (KSMFM) was found to be 24.05% and 22.08%, respectively. Sulfur percentage is more in KSCM as compared to KSMFM, indicating probably less evaporation in classical method because of less heat exposure in comparison to muffle furnace method. The action of KS is \textit{Muqawwie jigar} (hepatotonic), \textit{Muqawwie azae raesa} (tonic for vital organs), and \textit{Yarqan} (jaundice). Presence of sulfur also indicates regarding its utility because the quantity of sulfur in both classical and muffle furnace method was very high. Studied KS is indicated to improve the complexion of face, as a tonic for vital organs, and to increase blood production; action/mechanism of sulfur in this regard favors its action and uses. Role of sulfur in the formulation and its mechanism for its indication needs further evaluation.

### Table 5: Elemental analysis of \textit{Kushtae Shangarf} classical method (KSCM) and \textit{Kushtae Shangarf} muffle furnace method (KSMFM) (combined table)

| Elements          | Sample KSCM Method | Sample KSMFM Method |
|-------------------|---------------------|---------------------|
|                   | ICP-OES (ppm)       | ICP-MS (ppm)        | XRD Gravimetric (%) | ICP-OES (ppm) | ICP-MS (ppm) | XRD Gravimetric (%) |
| Mercury as Hg     | <0.1                | Not detected        | Phase not detected  | <0.1              | Not detected | Phase not detected |
| Arsenic as As     | 3.11                | 5098.14 ppm (0.509%)| Phase not detected  | 3.39              | 375.96 (0.037%)| Phase not detected |
| Iron as Fe        | 1146.3              | —                   | —                   | 1044.79           | —            | —                   |
| Lead as Pb        | <0.1                | —                   | Phase not detected  | <0.1              | —            | —                   |
| Cadmium as Cd     | <0.1                | —                   | Phase not detected  | <0.1              | —            | —                   |
| Aluminum as Al    | 57,482.87           | 66450.52 (6.645%)   | As \( \text{Al}_{2.66}\text{O}_4 \) | 23,133.72         | 28,376.67 (2.837%)| As \( \text{Al}_2\text{O}_3 \) |
| Calcium           | —                   | 3805.38 (0.380%)    | As calcium sulfide  | 1217.64           | 1217.64 (0.121%)| As CaS |
| Sulfur            | —                   | —                   | —                   | —                 | —            | 22.08               |

Microscopic study reveals very minor quartz and pyrite (1%–5%) in cinnabar (raw) and silicates in trace in alum (raw) used in both samples.
Findings of the study clearly suggest that in *Kushta* preparation of *Shangarf* prepared with 25 Ser (24kg) *Uple* heatput or simulated heat pattern in muffle furnace, no mercury was found in the finally obtained product not even in traces. We can conclude by this finding that all the *Kushta* prepared with excessive heat or put creating appropriate heat for facilitating evaporation of mercury can be safe. Evaporating/boiling temperature of mercury is near about 600°C.  

Use of this *Kushta* is mentioned “with butter or milk.” Generally, *Kushta* is given with oil, milk, or butter. These things have properties of detoxification and the property of removing of dryness (*Yabusat*). In some condition of arsenic toxicity and its dryness, milk and ghee can reduce their toxicity and save from severe adverse effect of arsenic toxicity and its dryness, milk and ghee can be a better option for preparing KS containing Jaiphal and Phitkari because of less quantity of arsenic found in the sample, low cost of preparation, controlled production, high output of *Kushta* (white part), and less chances of exposure to the fumes of cinnabar and mercuric sulfide. *Mizaj* (temperament) of *Shangarf* is mentioned generally as *Haar* (hot), *Yabis* (dry) in 2°, which cannot be categorized in a poisonous or highly toxic drug according to *Unani* concept.

**Conclusion**

As far as different processes of incineration are compared, it can be concluded that sample of KSMFM can be a better option for preparing KS containing Jaiphal and Phitkari because of less quantity of arsenic in the sample, low cost of preparation, controlled production, high output of *Kushta* (white part), and less chances of exposure to the fumes of Cinnabar. *Shangarf* incinerated above 900°C with Phitkari and Jaiphal did not show any presence of mercury in both the samples, clearly indicating that KS prepared by incinerating at higher temperature can be safer than unroasted or roasted (heated) preparation at lower temperature with respect to the presence of mercury, and it can be recommended or used for its indications without the toxicity concern. Further sophisticated evaluation with different temperature range and combinations can be carried out to find other elements on qualitative and quantitative basis with *in vivo* toxicity study so that more accurate assessment can be made regarding the toxicity of *Shangarf* preparations.

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**Conflicts of interest**

There are no conflicts of interest.

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