Standardization of *Kushta Sammul far* (Calx of Arsenic Trioxide) Prepared by Two Different Methods

[1]Athar Parvez Ansari,
[1]*Abdul Wadud,
[1]Najeeb Jahan,
[2]Shamim Irshad
and
[3]Uzma Jabeen

[1]National Institute of Unani Medicine, Kottigepalya, Magadi Road, Bangalore-560091

[2]Jamia Tibbia Deoband, Deoband, Saharanpur-247554 (U.P.)

[3]Dept of Microbiology, Govt. Science College, Bangalore University, Bangalore-560091

Abstract

This study was carried out on two samples of *Kushta Sammul far* (Calx of Arsenic trioxide), one prepared by classical method of calcination and the other one by muffle furnace in order to set the parameters for its standardization and to observe similarity or dissimilarity between the two samples if any, to offer a more refined alternate method of calcination. The parameters of standardization included tests for luster, fineness, floating, and curd and lemon tests substantiated with analytical methods which included determination of moisture content, pH value, particle size distribution; and Fourier Transform Infrared Spectroscopy (FTIR). The study revealed almost similar results in the two samples with slight difference in the physicochemical properties. The methods and the findings may be used as reference to prepare and standardize *Kushta Sammul far*.

Keywords: Unani Medicine, *Kushta*, Standardization, Particle size, F.T.I.R.

Introduction

Metals and minerals such as gold, silver, copper, iron, zinc, lead; salts, earthy matters and gems etc. are commonly used in traditional systems of medicine (Dubey *et al.*, 2008; Devanathan et al., 2010.) especially in Unani medicine and Ayurveda. Since, most of the above mentioned metals and minerals cannot be used systematically as it is, because of pharmacokinetic inconvenience and potential toxicity; therefore, strategies have been set for conversion of these metals and minerals into carbonate or oxide forms which can be used effectively and safely in small doses. The oxide is technically known as *Kushta* (calx), a term derived from a Persian word '*Kushtan*' meaning 'To kill" (Anonymous, 1997; Husain, 1940) indicating a process by which metals and minerals are killed i.e. burnt at high temperature.

Preparation of *kushta* (*s*) by classical methods is though time tested but the process is a bit unsophisticated, complex and time consuming, therefore it was found suitable to switch over to some alternate methods (Shamim, 2009). The possible and simple alternate is muffle furnace method provided the same pattern of temperature, as used in classical method, is extrapolated and adjusted in muffle furnace. Shamim *et al* (2009) also developed a thermogram by digitally recording the temperature pattern encountered during the preparation of *Kushta Sammul far* by classical method and extrapolated it on muffle furnace, and compared the *kushta (s)* prepared by these two

*Author for correspondence

methods with reference to acute and sub acute toxicity. They found almost similar results in the two samples. Since they have performed only animal study therefore it would not be appropriate to conclude that the two samples are similar in all respect. Despite being a well-established dosage form, and having wide scope of incorporation of modern techniques for validation of *kushta*, very few scientific reports on physicochemical properties and toxicity profile of *kushta* (*s*) are available. Therefore, in our study the two samples of *Kushta Sammul far* were subjected to the physicochemical standardization to see difference in physicochemical properties, if any. The parameters used for the purpose of standardization included test for luster, fineness, floating characteristics, change of colour, moisture content, pH value, particle size, polydispersity index and finally the Fourier Transform Infrared Spectroscopy.

Materials and Methods

Materials

*Arsenic***:** Arsenic trioxide (As_2O_3) was procured from Nice Pvt. Ltd., Kerala, India.

*Alum***:** Alum was procured from local market of Bangalore.

Preparation of Test Drugs

Two samples of *kushta Sammul far* were prepared**.** The sample prepared by the method described in National Formulary of Unani Medicine (Anonymous, 2006) named as KSCM, whereas the sample prepared by muffle furnace as per the method described by Shamim *et al*. (2009) was named as KSMF.

Standardization of Kushta Sammul far

For standardization the following methods were adopted.

(i) Classical Methods

(a) *Test for Luster*

KSCM and KSMF were taken in a Petri dish and observed for any luster in day light through magnifying glass (Anonymous, 2001; Mohapatra and Jha, 2010).

(b) *Test for Fineness*

KSCM and KSMF were taken in between the thumb and index finger, rubbed to see that the *Kushta* (*s*) have deposited into the lines of the finger and easily

washed out from the cleavage of the lines. (Anonymous, 2001; Mohapatra and Jha, 2010).

(c) *Test for Floating*

A small amount of KSCM and KSMF were sprinkled over the still water in a beaker to observe that the particles of *Kushta* (*s*) floated over the surface of water or not (Anonymous, 2001; Mohapatra and Jha, 2010).

(d) *Curd Test*

A pinch of KSCM and KSMF were mixed with a little amount of curd in a clean and dry Petri dish to observe any colour changes (Mohapatra and Jha, 2010).

(e) *Lemon Test*

A pinch of KSCM and KSMF were mixed with lemon juice in a test tube, to observe any colour changes (Mohapatra and Jha, 2010).

(ii) Conventional Methods

(a) *Determination of Moisture Content*

Moisture content was determined by loss on drying method. For estimation of moisture content, 200 mg of KSCM and KSMF were placed in known weight of porcelain dish separately in hot air oven at the temperature ranging 100 – 105 ^{0} C. The weight of the drug was continuously monitored after every two hour followed by cooling of drug in a desiccator, until the weight of the drug remained constant. The constant weight was then subtracted from the weight of the drug taken and the percentage of moisture was determined (Jenkins, 1940).

(b) *Determination of pH*

For pH determination, a Systronic digital pH meter (model 152- R) equipped with a combined electrode was used. The instrument was calibrated using buffer solution of 4.00, 7.00 and 9.20 to ascertain the accuracy of the instrument prior to the experiment. The test drug (100 mg) was taken to determine the pH in 1% solutions (Anonymous**,** 1968).

(c) *Particle Size Determination*

For particle size determination of the KSCM and KSMF, Dynamic Light Scattering (DLS) was used for the measurement of average hydrodynamic meters and Polydispersity Index (PDI). Each sample was analyzed in triplicate at 20°C at a scattering angle of 173. Pure water was used as a reference for dispersing medium. Zeta potential data were collected through electrophoretic light scattering at 25°C, 150 V, in triplicate for each sample (Malvern Zetasizer

Nano-1690, Malvern Instruments, UK) in pure water. The instrument was calibrated with Malvern-50 V standard before each analysis cycle (Paul et al., 2011).

(d) *Fourier Transform Infrared Spectroscopy (FTIR)*

Fourier Transform Infrared Spectroscopy of KSCM and KSMF was carried out by FTIR Spectrophotometer 8700 (Shimadzu). Potassium bromide (KBr) was used as a binder agent. Before making pellet, the KBr put in an oven for removing moisture content. Afterwards, *Kushta* and KBr were taken in the ratio of 2:100 (mg), triturated with the help of mortar and pestle and made pellet of 0.5 mm of thickness by pressing of KBr press. The prepared pellet was analyzed by FTIR Spectroscopy (Vikas *et al.*, 2009).

Results

Physico-chemical Standardization

(i) Classical Methods

Results of test for lustreness, fineness, floating, curd test, and lemon test are given in table 1.

(ii) Conventional Methods

Moisture content of KSCM and KSMF was as 0.77% and 0.55%, respectively (Table 01). The pH value of KSCM and KSMF was found to be 7.20 and 4.48, respectively (Table 01). The particles of KSCM were found to be 655.8 nm and that of KSMF 573.3 nm (Table 02; Figure 01 A & B). FITR analysis showed that functional group region of both samples presented with same peaks indicating the probable similarity in chemical properties of both the *Kushta* forms, but different peaks were seen in finger print region indicating variation in stereomeric configuration of the two *Kushta* forms (Figure 2 A & B).

Discussion

Though, most of the drugs used in Unani medicine are supposed to be safe, but some may be toxic even at therapeutic dose level and very few of them mainly metals / minerals may be highly toxic if not subjected to the process of detoxification, as described in Unani literature. The methods of detoxification may either be physical or chemical or a combination of both. Calcination, which is one of the physical processes of detoxification, aims at downgrading

the toxicity of the drug so as to make it safe for therapeutic application. But if the drug happens to be a metal it may still contain the element of toxicity. Earlier the physicians of traditional medicines were processing and preparing the medicines under their direct supervision therefore, the authenticity and the quality of drug was ensured, but now the situation has changed and drugs are mostly prepared by pharmaceutical companies and industrial houses. In such circumstance, it is necessary to setforth standard parameters for checking the purity and quality of mineral drugs.

When the KSCM and KSMF were subjected to classical methods of standardization, it was found that both samples were equitable and of desired quality. However, quantitative studies showed slight differences in the two samples.

Moisture content determination is a reliable parameter for assessing the quality of crude drugs, but estimation of moisture content in a drug which has been burnt at temperature exceeding 500^0C seems to be inconsequential, still in our study it was taken as a parameter because kushta being a fine powdery preparation and having larger surface area may absorb moisture during packaging and storage. The moisture content *of Kushta Sammul far* prepared by classical method should ideally not exceed 2% (Anonymous, 1986), which in our study was found to be 0.77% and 0.55%, in KSCM and KSMF, respectively indicating its good quality and also the reliability of the procedures adopted to prepare the two samples.

The pH of a drug may also be considered as an important parameter. Change in the pH of the solution can change the physical chemistry of a drug by adding or removing protons at certain sites of the molecule and thus increasing or decreasing the solubility of the drug (Anonymous, 2012). In our study the pH value of KSCM and KSMF was found to be 7.20 and 4.48, respectively showing difference in the pH of the two samples. The pH of KSCM inclined to alkalinity, whereas that of KSMF showed acidic nature. This difference might be because of some possible chemical changes occurred during the processing which rendered two different physical attribute to the two samples but it needs intensive investigation to ascertain the cause.

Particle size of drugs used for systemic effect influence their dissolution rate. It is therefore expedient to have small particle size of drugs as they will have larger surface area (Walter, 1994). Earlier, particle size of *kushta* (*s*) was judged on the basis of simple tests which were unable to give accurate results, but with the help of analytical methods it became possible to get the

size even in nm. Dynamic Light Scattering method is a good technique used to determine the size distribution profile of small particles. We found particle size of KSCM and KSMF estimated by Dynamic light scattering as 655.8 nm and 573.3 nm, respectively. Similarly, FTIR Spectroscopy is an important method for assessment of quality of crude drugs. Although, this technique is more useful for identification of functional groups present in organic compounds, but it was applied to our study material to observe difference, if any, in the spectra of the two samples. On comparing the FTIR spectra of KSCM and KSMF, we found same peaks in functional group region indicating similarity in chemical structure, but different peaks in fingerprint region were observed which may be due to some inequitable physical properties of the two samples. These findings indicated that KSCM and KSMF may have minor but insignificant difference in their physical and even in chemical properties. Since no reference literature on Dynamic Light Scattering and FTIR for any *Kushta* (*s*) including *Sammul far* is available for comparison therefore our study may be considered as one of the earliest reports in this area of research and may also be considered as standard for future references.

Table 1: Physical properties of *Kushta Sammul far* tested by classical methods

S. No.	Tests	Samples	
		KSCM	KSMF
1.	Test for Luster	–	–
2.	Test for Fineness	+	+
3.	Test for Floating	+	+
4.	Curd Test (color change)	–	–
5.	Lemon Test (color change)	–	–
	+ = Yes - = No		

Table 2: Moisture content, pH value and Particle size of KSCM and KSMF

S. No.	Samples	Moisture content (%)	pH	Particle size (nm)	Pdi
1.	KSCM	0.77	7.20	655.8	0.46
2.	KSMF	0.55	4.48	573.3	0.45

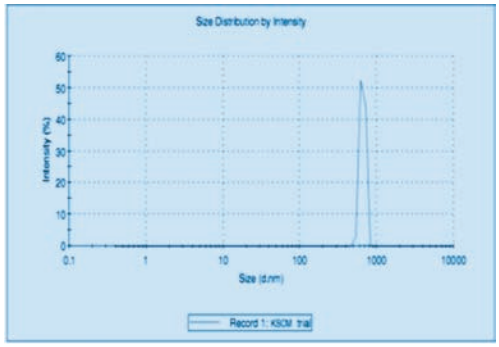

Fig.1: Particle size of KSCM

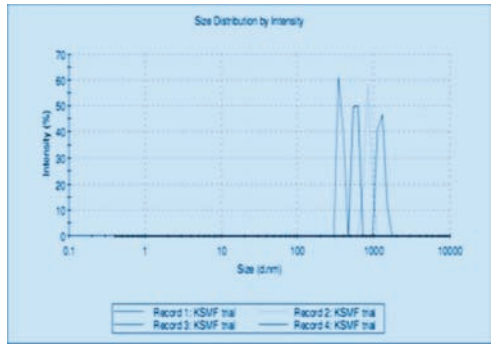

Fig. 2: Particle size of KSMF

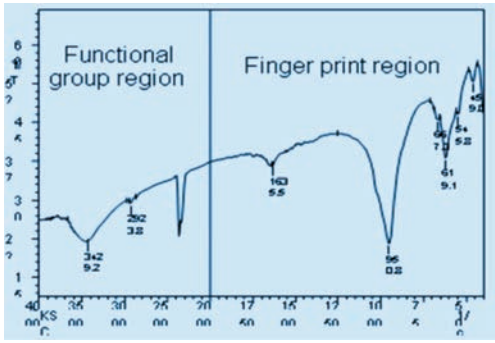

Fig. 3: FTIR spectrum of KSCM

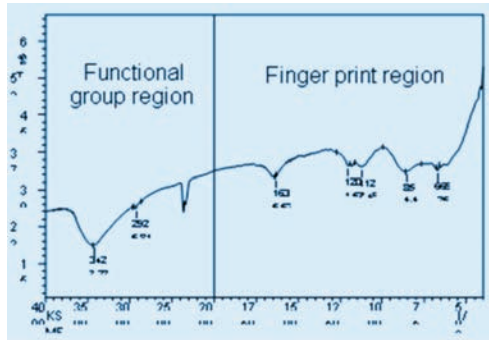

Fig.4: FTIR spectrum of KSMF

Conclusion

Our study sets the standard for the preparation and standardization of *Kushta* Sammul far. The methodology and the findings may be used to standardize the *Kushta* prepared from the drugs of mineral origin. The study also validated the use of alternate method of *kushta* preparation thus giving choice of using muffle furnace in place of relatively inconvenient conventional methods.

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