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Original Article

Temperature Standardization and Quality Standard of Kushta e Gaodanti by Adopting Classical and Modern Methods

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ABSTRACT

Background: *Kushta Gaodanti* (KG) is an essential Unani dosage form prepared by calcination according to classical texts. Standardization is paramount important to check purity and genuineness of the product. However, there are some drawbacks in the calcination process and assessing quality of the drug as per classical method. Hence, this study was aimed to evaluate the quality of KG by classical and modern methods and to develop a quality standard for KG. **Methods:** A multidisciplinary approach utilizing modern analytical techniques with classical tests was used. Thermogram developed when preparing KG by classical method was used to prepare the drug by Furnace method. The quality of both samples was evaluated on classical parameters like finger test, fineness test, floating test, grain test and wall test and modern physicochemical parameters like bulk density, tapped density, Hausner's ratio, Carr's index, pH, Loss on drying, ash values, and extractive values. Characterization using X-ray diffraction, Scanning Electron Microscopy, Energy Dispersive X-ray Analysis, Fourier-Transform Infrared spectroscopy, absorption spectroscopy, Zeta size and potential analysis, and heavy metal analysis have also been carried out to establish the quality standard of KG with modern analytical techniques. **Results:** The results of the tests done for both the preparations are comparable within experimental error when comparing with each other but non-significant differences were observed in certain parameters. **Conclusion:** The physicochemical and analytical parameters evaluated in this study may be considered as standard reference for KG. Furthermore, the thermogram developed in this study can be utilized to prepare the *Kushta Gaodanti* by the furnace method.

Key words: *Kushta Gaodanti*, Arthralgia, Unani Medicine, Gypsum, Physicochemical.

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INTRODUCTION

Unani drugs are mainly prepared from three different sources such as plant, animal and minerals. The drugs prepared with minerals and metals are considered to be superior when compared with plant and animal origin drugs due to various reasons. It is evident that some metal and mineral preparations have been used by ancient Unani physicians for certain debilitated diseases. In the Unani system of medicine, preparations containing minerals and metals prepared through a specialized calcination process is known as *Taklees*. The drug obtained through this method is known as *Mukallas* or *Kushta* (calcined drug).[1] In Ayurveda, it is known as Bhasma and Parpams in Siddha. The toxic drugs are converted into safe drugs and the therapeutic efficacy of the drug is enhanced by this calcination process.

Kushta Gaodanti is one of the herbo-mineral drugs prepared with gypsum (Gaodanti) and decoction of *Withania somnifera* (Asgand) through calcination process which is beneficial in arthralgia, numbness, hemiplegia, facial palsy, gout, fever, cramp, etc.[2] At present there are many herbo-mineral products available in the market but quality and effectiveness of these products are doubtful because of lack of quality and safety profiles. Preparation of *Kustha* by classical method is a very laborious and time-consuming procedure. Also, instability in the intensity of the fire, difficulty in controlling temperature and quality of cow dung cakes used to produce heat are some of the issues in the preparation of *Kushtajat*. These factors lead to fluctuation in the intensity of the heat results in batch-to-batch variation in the quality of the product. In order to overcome these issues modern equipment like furnace, oven and grinding machine could be utilized instead of classical grinding, drying and heating methods of calcination. Previously, the *Kusthajat* were prepared by the physicians themselves as per their requirements. Nowadays, they are manufactured in large scales in pharmaceutical companies. This new approach will create several problems such as adopting shortcut methods in the preparation, compromising the exact procedures, quality of raw material, etc. *Kushtajat* are safe when prepared and used in the proper way while prepared in inappropriate and short cut ways, may produce health hazards. Hence, standardization of *Kushtajat* is compulsory to get the quality product in order to get the desired benefits of the drug.

There are few methods and techniques mentioned in Unani classical texts to ensure the quality of the *Kushta* but those are highly subjective. Therefore, in this study along with classical tests advanced analytical techniques have been utilized to standardize the *Kushta Gaodanti*

to confirm its identity, determine its quality, purity and safety for the acceptability of the product.

MATERIALS AND METHODS

Raw materials

Gypsum and *Withania somnifera* were purchased from a local market in New Delhi. *Withania somnifera* is authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen No. NISCAIR/RHMD/Consult/2018/ 3307-08 is submitted in the herbarium of NISCAIR, New Delhi. Gypsum is identified and authenticated by the Department of Chemistry, Jamia Millia Islamia, New Delhi.

Preparation of *Kushta Gaodanti*

Kushta Gaodanti was prepared with 100 g of Gypsum and 100 ml decoction of *Withania somnifera* by classical method using cow dung cakes and furnace method.

Classical Method of Calcination

Kushta Gaodanti was prepared as per classical method described in National formulary of Unani medicine.[3] Gypsum was cleaned with hot water, and triturated in mortar and pestle with decoction prepared with 100 g of *Withania somnifera* until it became paste. Pellets were made from paste and dried well at room temperature. Next day, dried pellets were kept inside the two earthen vessels and sealed the mouth of the earthen vessels with mud plaster. A pit with one feet length, width and depth was dug in an open place. 50 kg of cow dung cakes were used to produce heat. Half the quantity of cow dung cakes was placed at the bottom of the pit. Then, the sealed apparatus (*Quza*) was placed in the center and the remaining cow dung cakes were kept over it and ignited. At the end of the process of calcination the pit was allowed to cool and the apparatus was removed from the pit. The burned pellets were removed from earthen vessels and made into powder with mortar and pestle. The whole process was repeated two times to prepare the final product. Likewise, three batches of *Kushta Gaodanti* were prepared and stored in airtight glass bottles. This preparation was termed as KGC. These samples were used to evaluate the quality standard of *Kushta Gaodanti*.

Temperature Standardization

A thermogram was developed by recording the temperature changes during calcination with the help of thermocouple digital pyrometer at an interval of 15 min at the entire course of preparation of *Kushta Gaodanti* by classical method. The recording of the temperature pattern was repeated three times and the average temperature pattern was utilized to prepare the *Kushta* by the furnace method.

Furnace Method

The same formula of *Kushta Gaodanti* prepared by classical method was used by the furnace method. Purified gypsum is ground with decoction of *Withania somnifera* in a double stone electrical grinder. Pellets were dried in the oven at low temperature and the dried pellets were kept in a closed crucible. Temperature was set in the tubular furnace as per the thermogram developed with the help of classical method of calcination. The whole process was repeated two times to prepare the final product. Likewise, three batches of *Kushta Gaodanti* were prepared and stored in airtight glass bottles. This preparation was termed as KGF.

Organoleptic evaluation

Colour, taste, odour and feel of KGC and KGF were observed.

Classical tests

The following methods mentioned in the Unani classical texts have been used to test the quality of KGC and KGF such as loss of metallic luster, fineness test, floating test, grain test, smoke test, and wall test.[4-6]

Modern Methods

Both KGC and KGF were evaluated for physicochemical tests like bulk density, tapped density, Hausner's ratio, Carr's index, pH, loss on drying, total ash, acid insoluble ash, water insoluble ash, water soluble ash and extractive values.[6]

X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), Fourier-transform infrared spectroscopy (FTIR), spectroscopic analysis and particle size and zeta potential determination have been carried out at central instrumentation facility, Jamia Millia Islamia, New Delhi.

UV-Visible spectroscopy

Spectroscopic analysis of the samples was carried out with dual beam Hitachi U3900 UV-Vis spectroscopy. 1 ml of aliquot was taken out from the solution and spectroscopic measurement was run at the wavelength of 250 to 650 nm.[7]

X-ray diffraction

The samples were analyzed by using Ultima IV X ray diffractometer, with CuKα radiation (λ = 1.541Å). The acceleration voltage was set at 30 KV and the current flux was set at 40 mA, while the Bragg angle was increased from 20 to 80 degrees.

Scanning Electron Microscopy and Energy Dispersive X-ray Analysis

The Zeiss EVO® MA10 (Carrl Zeiss, Oberkochen, Germany) SEM equipped with energy dispersive system (EDS XMax, 50 mm^2) controlled by Oxford Instruments Anal System (Oxford Instruments, Oxford, UK) was utilized to study the morphology and elemental composition of samples at a resolution of 1024 x 884 Mag:5000 x HV: 20.0 KV.

Fourier-Transform Infrared Spectroscopy

Bruker's Tensor 37 spectrometer was used for FTIR scanning of samples in attenuated total reflectance (ATR) mode. FTIR was carried out with 5 mg of powder samples with constant nitrogen purging in transmission mode. Average 16 scans in the range of 400-4000 cm^{-1} at 4 cm^{-1} resolution had been utilized to take all the spectra.

Particle Size and Zeta Potential Determination

Samples at the concentration of 1mg/ml in distilled water were analyzed for the particle sizes and the zeta potentials by using dynamic light scattering and electrophoretic light scattering with a Zetasizer, Nano ZS (Malvern Instruments Limited, UK) respectively at 25°C.

Heavy Metal Analysis

Heavy metal analysis of Gypsum, KGC and KGF samples were done by atomic absorption spectroscopy method according to protocol for testing of AUS medicine.

RESULTS

A thermogram developed by recording the temperature pattern of *Kushta Gaodanti* prepared by classical method using cow dung cake is depicted in Figure 1. It was observed that the temperature increased rapidly up to 950°C within 2 ½ hr and then decreased steadily over 5 ½ hr. Afterwards the temperature remained below 100°C for more than 12 hr.

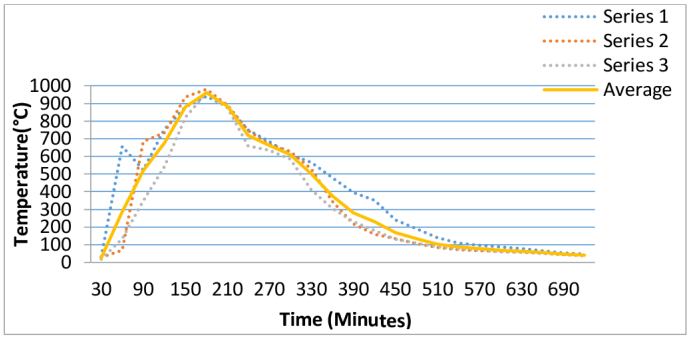

Figure 1: Temperature pattern in the preparation of *Kushta Gaodanti* by classical method.

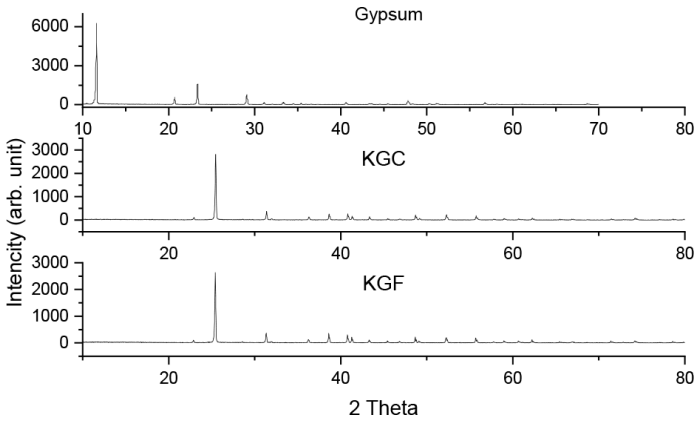

Figure 2: XRD of gypsum, KGC and KGF.

Table 1: The results of physico-chemical analysis.

S.No	Parameters	Results	
		KGC	KGF
A	Bulk Density	0.87 g/ml	0.91 g/ml
B	Tapped Density	1.43 g/ml	1.6 g/ml
C	Hausner's Ratio (HR)	1.64	1.76
D	Carr's Index	39.13	43.18
E	pH of 1% solution	9.91	8.71
F	Loss on drying	0.22 %	0.21 %
G	Ash Value		
a.	Total ash	99.95 %	99.96 %
b.	Acid insoluble ash	88.80 %	87.87 %
c.	Water insoluble ash	98.80 %	98.025 %
d.	Water soluble ash	1.08 %	1.925 %
H	Extractive value		
a.	Methanolic extract	0.21 %	0.17 %
b.	Aqueous extract	1.42 %	1.24 %

The developed thermogram was utilized to prepare *Kushta Gaodanti* by the furnace method.

The organoleptic evaluation of the both test drugs (KGC and KGF) showed tasteless, odourless, grey in colour and smooth. The results of tests to assure the quality of perfectly prepared Kushta mentioned in the Unani classical text such as loss of metallic luster, fineness test, floating test, grain test, wall test were positive and the smoke test was negative for both samples. The results of physico-chemical tests of samples are summarized in Table 1. The heavy metals analysis showed that Mercury, Arsenic and Cadmium were not detected in all three samples, while Lead was detected in Gypsum (4 ppm), KGC (6.2 ppm), and KGF (6.1 ppm).

Results of XRD of gypsum, KGC and KGF are presented in Figure 2. The 2θ value corresponding to the highest intensity (6272 arb. unit) in gypsum is 11.6 whereas in KGC (2827 arb. unit) and KGF (2646 arb. unit) are 25.46 and 25.42 respectively. The element/molecule for the corresponding 2θ and intensity of the peaks and their relevant JCPDS files numbers are presented in the Table 2. The absorbance spectra of gypsum, KGC and KGF are depicted in Figure 3 which shows maximum absorption at 228 nm in the UV region.

FTIR of gypsum, KGC and KGF are illustrated in Figure 4 and functional groups matching with the peaks obtained in FTIR of all the samples are shown in Table 3. The Scanning Electron Microscopy (SEM) was carried out to analyze the particle size of KGC and KGF. The particle size of KGC ranges from 20 nm to 7 µm while in KGF, it ranges from 40 nm to 8 µm (Figure 5). The EDAX analysis of Gypsum, KGC and KGF revealed that the chief elements are Ca, S, O, C and their composition in different samples are shown in Table 4. The Table 5 shows the range of particle size, maximum particles size, average particle size, average zeta potential, zeta deviation, and conductivity of KGC and KGF.

DISCUSSION

Kushta is an essential dosage form of the Unani system of medicine. It is rapidly absorbed in the human body due to the small particle size and leads to instant therapeutic actions. Though the *Kushta Gaodanti* is prepared by a specialized process called calcination according to methods mentioned in the classical texts, there are some drawbacks in the procedures. The main issues in the preparation of *Kushta* by classical method are instability in the intensity of the fire, difficulty in controlling temperature and quality of cow dung cake is not consistent. This can be overcome by utilizing modern equipment like furnace which have effective mechanisms to regulate the temperature. Further, the traditional way of grinding and drying consumes more time than using modern electrical laboratory oven and grinding stone.

In present study, *Kushta Gaodanti* was prepared by classical method and a thermogram was developed by recording the temperature changes during calcination to prepare the drug by Furnace method. The thermo gram developed in this study can be utilized as a standard temperature pattern to prepare the *Kushta Gaodanti* by Furnace method. Also, the tests to determine the quality of *Kushtajat* mentioned in Unani classical texts are highly subjective. Hence, in this study, quality standards of Kustha Gaodanti were developed utilizing classical and modern analytical methods.

Properly prepared *Kushta* should not have metallic luster and when a pinch of *Kushta* rubbed between thumb and index finger the powder should enter into finger creases (Fineness test). When sprinkle the *Kushta* on cold and still water, it should float on the surface (Floating test). Rice grain should not sink when it is placed slowly on floating *Kushta* if it is the best quality (Grain test). Properly prepared *Kushta* should not emit smoke when it is burned (Smoke test) and when sprinkled a small amount of *Kushta* on the wall, finely prepared one will stick to the wall (Wall test).[5] As per the results the two samples passed the quality tests as stated in the Unani classical texts. The physico-chemical tests such as pH, loss on drying, ash values, and extractive values are comparable within experimental error in all two samples, but some non-significant differences are observed in certain tests like bulk density, tapped density, Hausner's ratio and Carr's index (Table 1).

Table 2: Molecule and their corresponding JCPDS reference number, 2θ value and intensity of gypsum, KGC and KGF.

Molecule	JCPDS Ref. No	2θ (Intensity)		
		GYPSUM	A	B
$CaSO_3(H_2O)0.5$	84-0962		38.66 (260)	38.6 (360)
$CaSO_4(H_2O)0.67$	85-0531		41.4 (186)	
$Ca(HSO_4)2$	85-1271		22.9 (127)	22.9 (115)
$Ca(SO_4)(D_2O)2$	86-0945	11.6 (6272)		
		20.7 (535)		
		29.1 (764)		
	86-0943		31.38 (375)	31.34 (381)
	86-0945		36.32 (144)	36.28 (137)
$CaAl_3(PO_4)(SO_4)(OH)_6$	87-0600		40.82 (270)	40.78 (300)
(Ca. Na, La, Gd)10(P, S)6O24(F, OH)2	87-2026		25.46 (2827)	25.42 (2646)
$Ca[(H_SO_4)2(H_2O_4)2]$	88-0764			41.28 (228)
$Na6CaMg(IO_3)6(CrO_4)_2(H_2O)_{12}$	88-1219	23.4 (1586)		
		31.1 (170)		
		40.6 (205)		
		47.8 (311)		
$Ca[Zn_8(SO_4)2(OH)12C_{l2}](H_2O)9$	89-0851	33.3 (209)		
$Ca(SO_4)(H_2O)0.662$	89-1445		43.34 (144)	43.3 (127)
			52.30 (228)	52.28 (198)
				62.24 (127)
$(Na, Ca)4(Al_7Si)12\ O_{24}\ (Cl, CO_3, H, SO_4)$	89-7419		55.74 (197)	
$Ca0.857\ Na0.285\ (SO4)(H_2O)0.473$	89-8618	56.8 (170)		
	89-8619		48.68 (218)	48.66 (218)
$Ca\ [(NH_2)\ SO_3]2(H_2O)4$	89-9106			55.68 (198)

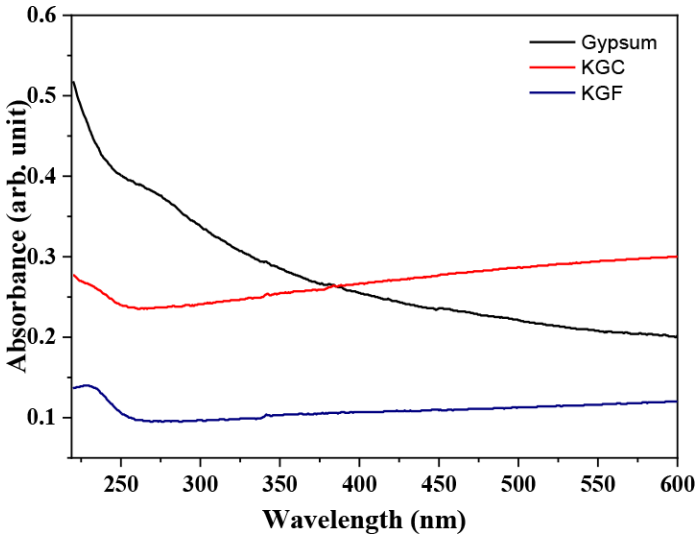

Figure 3: Absorbance spectra of gypsum, KGC and KGF.

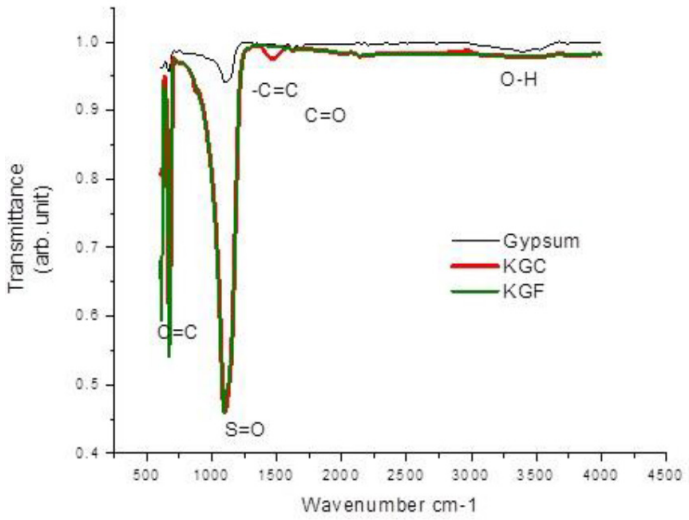

Figure 4: FTIR of gypsum, KGC and KGF.

The results of heavy metal analysis of Gypsum, KGC and KGF are found to be within the permissible limits as per the World Health Organization (WHO) permissible limits for heavy metals.

When comparing the molecules in the gypsum and the *Kushta Gaodanti* prepared by classical and furnace methods, new molecules are identified in the KGC and KGF. Further, almost similar compounds are present in the KGC and KGF (Table 2). Different molecules absorb radiation of different wavelengths in UV-Visible absorption spectroscopy in the ultraviolet-visible spectral region (UV range 150 - 400 nm and visible range 400-800 nm).[8] The absorption spectrum produced by this shows the absorption bands corresponding to the structural groups present in

Table 3: Functional groups of gypsum, KGC and KGF.

S.No.	Functional group	Peak		
		Gypsum	Sample A	Sample B
1	Strong C=C bending alkene	665.3907	675.074	675.84
2	Medium C=C bending alkene trisubstituted	796.59		
3	Strong S=O stretching sulfonic acid	1100.67	1098.86	1098.86
4	C–H bend alkanes		1460.08	
5	Strong N-O stretching nitro compound	1537.64		
6	Medium C=C stretching cyclic alkene	1625.86		
7	Strong C=O stretching conjugated acid dimer	1684.91		
8	Weak CΞC stretching alkyne disubstituted	2204.46		
9	Medium CH stretching			
10	Strong broad O-H stretching alcohol inter molecular bonded	3390.31		
11	Strong broad O-H stretching alcohol inter molecular bonded	3525.76		

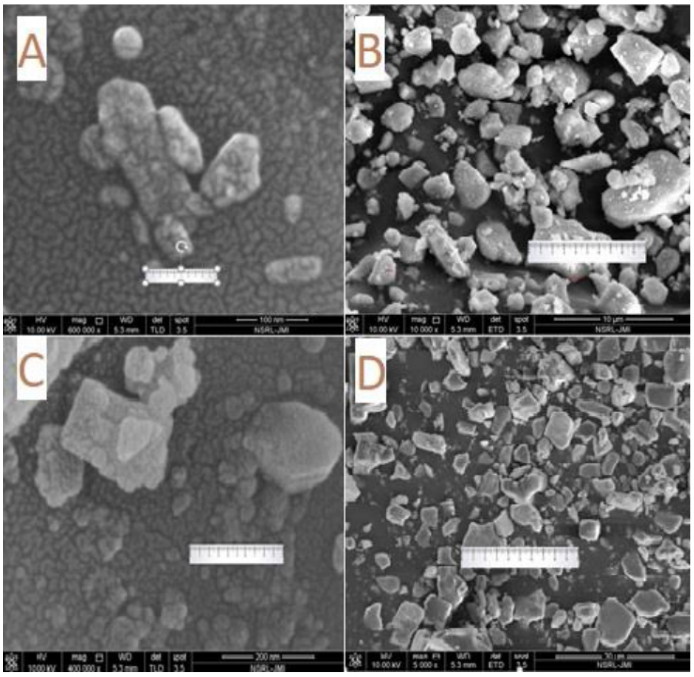

Figure 5: Scanning Electron Microscopy. (A) KGC (X 600,000); (B) KGC (X 10,000); (C) KGF (X 600,000); (D) KGF (X 10,000).

the molecule. Though, there are differences in the absorbance spectra of gypsum, KGC and KGF (Figure 3), the maximum absorption value is same for all three samples (228 nm in the UV region).

Energy transfer takes place in the manner of electron ring shift, molecular bond vibrations, rotations and stretching of bonds. The principle of FTIR

Table 4: EDAX analysis of Gypsum, KGC and KGF.

Elements	Gypsum C Atom. [at.%]	KGC C Atom. [at.%]	KGF C Atom. [at.%]
O	3.0	61.58	61.88
Ca	23.31	13.63	14.23
C	3.8	12.66	12.70
S	18.15	12.13	11.19

Table 5: Zeta size and potential values of KGC and KGF.

Sample	Range of Particle size (nm)	Range of Maximum Particles size (nm)	Average Particle Size (nm) (Z average)	Average Zeta Potential (mV)	Zeta Deviation (mV) (SD)	Conductivity mS/cm
KGC	≤1–8555	395.67 ± 53.50	1298	-8.95	1.54	0.0212
KGF	≤1–8600	542.6 ± 125.05	1827	-6.00	0.34	0.0218

is based on the molecular movement to higher states by absorbing the light in the IR range. FTIR spectra of a sample is analyzed as a function of wavelength or wavenumber of incident radiation. The FTIR spectra gives valuable information about the structure of the particular molecules via appearance and nonappearance of certain vibrational frequencies. As each functional group has unique vibrational frequencies the FTIR spectra also gives the information on the existence of certain functional groups in the sample for their characterizations.[9]

According to the FTIR spectrum it can be assumed that presence of functional group S=O (strong) is more prominent in KGC and KGF than gypsum while new functional group C–H bend has been formed in KGC and the O-H which was present in the gypsum is completely disappeared in both the *Kushtajat* (KGC and KGF).

As seen in Figure 5, particles are heterogeneous and in nano size. Therefore, these drugs have increased rate of absorption and quick effects on the tissues. The EDAX analysis of Gypsum, KGC and KGF revealed that the chief elements are Ca, S, O, and C. The percentage of Calcium (23.31%) and Sulphur (18.15 %) were greater in raw Gypsum than in two *Kushta e Gaodanti* preparations. The Calcium and Sulphur in KGC are 13.63 % and 12.13 % while in KGF 14.23 % and 11.19 % respectively. The highest element is O in Kushta Gaodanti that is 61.58 % in KGA and 61.88% in KGF. The percentage of C element is very low in raw gypsum (3.8 %) and it was increased to 12.66 % and 12.70% in KGA and KGF respectively.

The particle size of the drug is a key factor in determining its therapeutic efficacy in the body by rapid absorption and dispersion. The range of maximum particle size of KGC and KGF are 395.67 ± 53.50 nm and 542.6 ± 125.05 nm respectively. This shows that the procedures carried out during the calcination process are responsible for the reduction of the size of particles. Further, nano size of the samples is reason for quick absorption and enhance therapeutic efficacy in the body. Zeta potential is an electrical potential between particles to identify the colloidal stability of the substance. Substances with high zeta potential are having good stability and easy dispersion while substances with low zeta potential are vice versa.[9] The Zeta Potential (mean) value of KGC (-8.95 mV) and KGF (-6.00 mV) are found to be very low which indicate aggregation and less stability of particles in the suspension. Furthermore, KGC is more stable than KGF.

CONCLUSION

This study reveals multidisciplinary approach utilizing modern analytical techniques with classical tests would be more suitable in assessing the quality of *Kushtajat*. The results of physicochemical tests of *Kushta Gaodanti* prepared by classical cow dung cake method and modern furnace method are comparable within experimental error when comparing with each other but non-significant differences were observed in XRD, SEM, EDAX, FTIR, UV absorption spectroscopy, Zeta size and potential analysis. Therefore, by using advanced analytical techniques this work grants an applicable correlation between the traditional information on characterization and processing of Kustha. Further, the physicochemical and analytical parameters evaluated in this study may be considered as standard reference for *Kushta Gaodanti* to give safe and effective medication for mankind.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

KGC: *Kushta Gaodanti* was prepared by classical method; **KGF:** *Kushta Gaodanti* prepared by furnace method; **XRD:** X-ray diffraction; **SEM:** scanning electron microscopy; **EDAX:** energy dispersive X-ray analysis; **FTIR:** Fourier-transform infrared spectroscopy.

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