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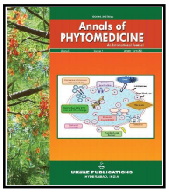

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Development of standard operating procedures and quality standard of Kushta Gaodanti with HPTLC fingerprinting and hyphenated techniques

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Abstract

Kushta Goadanti is a compound Unani formulation, prescribed in Unani System of Medicine for therapeutic actions, such as Daf-e-Tap (Antipyretic), Mohallil-e-Waram (Anti-inflammatory) and therapeutic used such as In Waja-ul-Mafasil (Arthralgia), Niqras (Gout) and Irq-un-Nisa (Sciatica) has been taken up for standardization by modern hyphenated techniques, so as to ascertain its quality standards. In the present study, Kushta Gaodanti was prepared by classical methods and standardization, was carried out in the premises of Department of Ilmul Advia and approved by the Institutional Ethical Committee (NTC/A/16/101), Government Nizamia Tibbi College, Hyderabad and the modern hyphenated techniques were studied in the laboratory of CSIR-IICT (DW 0560), Tarnaka. In this study, Kushtae Gaodanti was prepared by three methods. The Kushta Gaodanti prepared was analyzed through organoleptic properties, preliminary tests and physicochemical parameters, HPTLC fingerprint study along with hyphenated techniques such as XRD studies for the metal analysis in the samples. Moreover, elements and heavy metals were also estimated in all the three samples of Kushta Gaodanti. The data and results obtained in the study are thoroughly described in the paper. The parameters such as physico-chemical parameters, high performance thin layer chromatography (HPTLC) and XRD studies which are carried out, revealed as the specific identity for the drug under study and to establish as a pharmacopoeial standards. Results suggested that the drug is safe for therapeutic use and its batch-to-batch identification and determination of quality can be checked using the present study as reference standard in future.

1. Introduction

Kushta term refers to the finest powder form of medicinal preparations, obtained through the calcinations of metal, mineral and sometimes includes animal origin drugs. The raw drugs were processed by a special process called calcination carried in closed crucibles and also placed in pits of different sizes, bedding with the several numbers of cow dung cakes and with different intensity of heat produced through cow dung cakes. Kushta (calcined product) is easily absorbed in the human body and is highly effective in its action. Characteristics tests for properly prepared kushtajat are as there should be no metallic luster, when Kushta taken between the index finger and thumb and spread, it should be as fine as to get easily into finger lines. When a small quantity of Kushta is spread on cold and still water, it should float on the surface. The genuine Kushta prepared through the correct procedure of calcinated should not revert back to the original state.

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WHO has emphasized the need to ensure quality control of herbal based products by using modern techniques and by applying suitable parameters and standard. To overcome certain inevitable shortfalls of the Pharmacopoeial monograph and quality control measures, there is a need of research in this direction (Pifferi *et al*., 1999; street *et al*., 2008; Shinde, 2009; Singh and Soni, 2004).

The safety, efficacy and purity of compound herbal medicines are mainly depend on the quality which directly reflects the pharmacological properties of the medicinal product. To establish the standard, only organoleptic parameters like colour, odour, taste, *etc*., will not be sufficient to establish the quality standard of the medicine but it requires to thoroughly study by modern hyphenated techniques to generate evidence based scientific data.

Gypsum is composed of calcium sulphate (very soft sulphate mineral). It is found in Alabaster, as a decorative purpose used in India (Kabeeruddin, 2007; Ali, 1979) and ancient Egypt. It is widely used in the manufacturing of plaster ingredients like surgical splints, casting moulds, modeling, impression plasters in dentistry. Gypsum yields water when heated and becomes white and opaque.

Kushta is a dosage form in use since ancient times in classical text of Unani literature, as it is highly effective in prevention and cure of various diseases. Unlike other formulations, preparation of

Kushta following all classical precautions is very laborious and time consuming procedure. Preparation of Kushta entails heavy metals contamination and even those elements which are otherwise considered as toxic and not administered internally to conventional medicine. However, these materials before subjecting to calcination, is purified to get rid of impurities and toxic effects. Various detoxification and Kushta preparation methods are mentioned in classical texts, which are still in practice. Till date standardization of detoxification, standardization of heat process on scientific parameters are deficient, to convince the scientific community. Moreover, the efficacy of the drug mainly depends upon its physical and chemical properties. Therefore, the study of physicochemical, standardization (Rasheed, 2013; Rasheed *et al*., 2014; Rasheed *et al*., 2012 and Rasheed *et al*., 2017) is absolutely necessary in quality control of Kushtajat.

There are nearby thirty chemical elements that have their vital importance in various biochemical and physiological mechanisms in living organisms and considered as essential elements of life. Heavy metals also have great impact on human health and even those considered as essential can be toxic, if they are present in excess. Generally, humans are exposed to these metals by ingestion and inhalation. The term 'heavy metals' refer to any metallic elements that has a relatively high density and are causing toxic effects or poisonous even at low concentration. "Heavy metals" in general applies to the group of metals and metalloids with atomic density greater than 4 gm/cm, or 5 times or more, greater than water. Most common heavy metals are Lead (Pb), Arsenic (As), Cadmium (Cd), Nickel (Ni), Cobalt (Co), Zinc (Zn), Chromium (Cr), Iron (Fe), Silver (Ag) and the platinum groups (Dubey *et al*., 2008).

Therefore, in the present study, an attempt has also been made to estimate the heavy metals and other important elements present in the three samples of Kushta-e-Gaodanti.

However, the traces or little amount of heavy metals are basically present in some of the simple and compound Kushta formulations of Unani medicine which are being used for centuries. Unani compound formulation like Kushta, contain toxic elements/metals. That is used as detoxified after various purification processes like soaking, quenching, trituration, incineration, *etc.* Classical Unani texts have elucidated several detoxification processes for this purpose by various methods like using herbal pulps, extracts, *etc.* Hence, metals in the finished products may not possess toxicity. Though, such purification processes have been carried out by the Unani physicians, in accordance with described methodology, but these methods should be validated on experimental basis to the claims especially in reference to study the change and reduction of the toxicological symptoms due to their toxicity.

A polyherbal formulation, Kushta Goadanti is a popular drug which is widely used as an antipyretic, anti-inflammatory medicine taken for the present study is a Unani compound formulation mentioned in National formulary of Unani medicine of India, Part-V (Anonymous, 2008). The main ingredient of the formulation is Hartal Gaodanti or Gaudanti which means Gypsum or hydrated calcium sulphate ($CaSO_4$. $2H_2O$). The drug is prescribed in Unani system of medicine for actions such as Daf-e-Tap (Antipyretic), Mohallil-e-waram (Anti-inflammatory) and therapeutic used such as In Waja Mafasil (Arthralgia) (Zillurahman, 1985); Niqras (Gout) and Irq-un-Nisa (Sciatica). It is used in multiple systemic disorders like Muhallil-e-waram (inflammation), Hummiyath (Pyrexia), Falij (Haemiplegia), Waj-ul-mafasil (Rheumatism) (Tariq *et al*., 2013; Khan, 2012; Anonymous, 2009a; Anonymous, 2004; Anonymous, 2007; Gani, 1998; Kulkarni, 1991; Nadkarni, 1976; Ibn Baitar, 1999). A various classical process of detoxification and preparation of Kushta gaodanti mentioned but to establish a pharmacopoeial reference standard, an attempt had been made out in the present study. The study is aimed to evaluate the differences, if any in the various samples of Kushta Gaodanti prepared by different methods of detoxification.

2. Materials and Methods

Collection of material

Gaodanti, Gheegawar (*Aloe barbadensis* Mill.), Asgand (*Withania somnifera* (L.) Dunal) (Figure 1) was procured from the local herbal drug supplier, authenticated and identified before using. It was cleaned thoroughly to remove any foreign matter present on it.

Figure 1: Raw drugs-Gaodanti and Asgand.

Preparation of the formulation: NFUM. V, p 54-(Anonymous, 2008)

It is prepared according to the composition of the formulation as follows:

Formula 1: **KGF1**	Gaodanti	Calcium sulphate	100 g.
	Gheegawar	Pulp of *Aloe barbadensis* Mill.	100 g.
Formula 2: **KGF2**	Gaodanti	Calcium sulphate	100 g.
	Joshanda-e-Asgand	Decoction of roots of *Withania somnifera* (L.) Dunal	100 ml
Formula 3: **KGF3**	Gaodanti	Calcium sulphate	100 g.
	Sufoof-e-Asgand	Powder of roots of *Withania somnifera* (L.) Dunal	100 g.
	Gheegawar	Pulp of *Aloe barbadensis* Mill.	100 g.

The present study, Kushta Gaodanti was prepared by classical methods and standardization was carried out in the premises of Department of Ilmul Advia and approved by the Institutional Ethical Committee (NTC/A/16/101), Govt. Nizamia Tibbi College, Hyderabad, India and the modern hyphenated techniques were studied in the laboratory of CSIR-IICT (DW 0560), Tarnaka, Hyderabad, India. In this study, Kushtae Gaodanti was prepared by three methods are as given below:

Formula 1: KGF1 as per Qarabadeen-e-Majeedi (Anonymous, 1986)

The ingredients of Khusta Goadanti as described above are taken as described in the pharmacopoeia and ingredients are cleaned and made free from any foreign materials. Gaodanti was cleaned with hot water dried and 100 g of purified Gaodanti was powdered using mortar and pestle. Add 100 ml pulp of gheegawar and grinded in mortar for 4 h and made into paste. The obtained paste made into pellets (Anonymous, 1986) (Figure 2) and dried at room temperature for one day. A pit was dug about one and half feet and pellets were place between the cowdungs and covered with clay (Gil-e-Hikmat) and subjected to ignition to dry completely. Next day morning, the cowdungs were removed and the pellets burned out where collected powdered separately in mortal and pistle and stored.

Figure 2: Pelletes preparation for Khusta preparation.

Formula 2: KGF2 as per National Formulary of Unani Medicine (Anonymous, 2006).

Roots of *Withania somnifera* (Ashwagandha) was taken of about 100 g and soaked in 200 ml of warm water till night. Next day, boil the mixture and decoction was collected and filtered. Gaodanti was cleaned with hot water dried and 100 g of purified Gaodanti was powdered using mortar and pestle. The filtered Asgand decoction was added to Gaodanti powdered and triturated till it turns into paste. The obtained paste made into pellets and dried at room temperature for one day. After this, the same procedure was followed for the pellets as mention in formula 1.

Formula 3: KGF3 as given in The Text Book of Unani Pharmacy. (Parwaiz and Parveen, 2012) Take Gaodanti and Asgand roots of pharmacopoeial quality and clean the ingredients. Gaodanti was cleaned with hot water dried and 100 g of purified Gaodanti was powdered using mortar and pestle. In an earthen pot, the Gaodanti powder was placed in such a way that the Gaodanti powder was between the layers of Asgand powder. Then add 100 ml of pulp of gheegawar to it and seal the earthen pot with clay (Gil-e-hikmat). A pit fill with 20 kg cowdung cakes and earthen pot was kept over it and ignited. Next day morning, the inside powdered material was removed finely powdered using mortar and pistle and stored in air tight closed glass container.

Experiment

The Kushta Gaodanti prepared in three different methods: i. Kushta Gaodanti grounded in gheegawar pulp (KGF1), ii. Kushta Gaodantiprepared in decoction of asgand (KGF2), and iii. Kushta Gaodanti prepared with asgand powder and gheegawar pulp (KGF3) and all the three samples were evaluated for differences in the organoleptic, physicochemical, HPTLC, XRD properties. Physicochemical parameters such as particle size, total ash, acid insoluble ash, pH, bulk density, loss of weight on drying at 105 °C, *etc.*, were also carried out.

Kushta Gaodanti has different functions, depending upon the method of preparation. So, far the physicochemical characteristics of Kushta Gaodanti have not been previously evaluated on scientific parameters. Therefore, the present study was aimed to prepare Kushta Gaodanti by detoxifying Gaodanti in two different media and one in both media to observe and document the changes, occurring after calcinations and inscribe the physicochemical properties of the finished product. In the present study, preparation process of Kushta Gaodanti in various texts of Kushta sazi where the purification process is same in all texts triturating with different plant material extracts. Many texts mention multiple uses of Kushta Gaodanti prepared by *Aloe vera* juice and asgand decoction, and in both. NFUM also mentioned scientifically. Hence, all the three methods were taken as standard and try to evaluate the changes, if any.

In order to standardize and to lay down the standard operating procedures (SOP's) and pharmacopoeial standards, the formulation was prepared in three different methods at laboratory scale. It was subjected to analysis for organoleptic parameters, physicochemical parameters, TLC, high performance thin layer chromatographic (HPTLC) studies (Anonymous, 2009b; Naikodi, *et al*., 2011). The present paper describes the salient features of preparation, organoleptic parameters, phytochemical screening, safety evaluation studies and high performance thin layer chromatographic studies for the drug.

Organoleptic characters: Prepared Kushta was evaluated for colour, odour, taste and luster characteristics.

Preliminary test

i. Floating test: Small quantity of Kushta was sprinkled on water and observed if it floats on the surface or not.

ii. Fineness test: Fineness and smoothness were recorded by rubbing a small quantity of Kushta between the index finger and thumb and noted whether it deposits into crease and lines on ventral aspects of fingers.

iii. Loss of metallic lustre: Kushta was examined for metallic luster with naked eye in sun light.

iv. Wall stick test: Kushta was also examined by throwing on the wall to check whether it stick to the wall or not.

v. Bulk density and tapped density: 10 g of weighed Kushta was carefully added to the cylinder with the aid of a funnel. The initial volume was noted and the sample was then tapped until no further reduction in volume was observed. The bulk and tapped densities were calculated by the formula.

vi. Loss of weight on drying at 105 °C: 200 mg of Kushta was spread uniformly in petridish and was heated at 105 °C then cooled in a desiccator and weighed. The process was repeated till two consecutive weights were constant. The per cent loss in weight was calculated.

vii. Determination of pH in 1% solution: The pH value of 1% solution in accurately weighed 1 gm of Kushta was dissolved in accurately measured 100 ml of distilled water and filtered with whatman filter paper. pH was measured using a digital pH meter.

viii. Total ash: 2 gm of Kushta was incinerated in a silica crucible at a temperature not exceeding 450 °C. The crucible was then cooled and weighed and the percentage of total ash was calculated.

ix. Acid insoluble ash: The ash was boiled with 25 ml of dilute hydrochloric acid for 5 min. The insoluble matter or ash which was collected or left behind on filter paper (ash less) was washed with hot water and then subjected to ignition at a temperature not exceeding 450 °C and weighed after cooling to room temperature. The percentage of acid insoluble ash was calculated.

x. Water insoluble and water soluble ash: The ash was boiled with 25 ml of distilled water for 5 min. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited. The weight or amount of insoluble ash obtained was subtracted from the weight of the total ash obtained, which provides the resultant weight of the water soluble ash.

HPTLC analysis

Preparation of chloroform extract for HPTLC analysis

Five grams fine powder of KGF1, KGF2, KGF3 was placed in a conical flask and 100 ml of chloroform was added and kept on shaker for 8 h. Flasks were removed and the contents were filtered through Whatman No. 41 filter paper and evaporated the resultant solution to become 20 ml. Thus, the solution so obtained was used for HPTLC.

Development and determination of the solvent system

The sample extracts applied was about 10 µl and the mobile phase system was used as 100 % hexane. The sample was spotted with the help of automatic TLC applicator on the precoated aluminium sheets of silica gel 60 F_{254} (Merck) and developed in the TLC chamber to develop the TLC plate.

Development of HPTLC technique

The TLC plate after developing was removed and dried completely and detected under with the suitable detection system, Ultraviolet Cabinet system for detection of spots at λ=254 nm as shown in the Figure 3. Further, it was scanned with the densitometer under the UV range of λ=254 nm as shown in the Figure 4.

Metal analysis

In this study, elemental analysis was carried out by Energy Dispersive X-Ray Fluorescence (CSIR-NGRI), XRD Analysis (CSIR-NGRI), FTIR (CSIR IICT), Thin Layer Chromatography (CSIR IICT).

Energy dispersive X-ray fluorescence (EDXRF)

Non-destructive studies were carried out at the Council of Scientific and Industrial Research and National Geophysical Research Institute (CSIR-NGRI) facility. The EDXRF spectrometer (Epsilon 5: PAN analytica, Netherlands make) used in the present study-equipped with a ScW anode X-ray tube with the goal of increased sensitivity for lighter as well as heavier elements. The instrument has a series of user selectable secondary targets and is equipped with a liquid-nitrogen-cooled Ge solid-state high-resolution detector with a Be (8 lm) window. A 3-D design (or Cartesian geometry) was adopted for the instrument to eliminate the X-ray tube spectrum by polarization. Consequently, the background can be an order of magnitude lower than the traditional 2-Doptics, resulting in much lower detection limits. The EDXRF used in the present study is equipped with a 100 kV Sc/W tube, even heavy rare earth elements (from Eu to Lu) can be analyzed using the more sensitive "K" lines and, hence the instrument is less susceptible to spectral overlapping with the K lines of lighter elements. The samples were analyzed using the pressed powder pellets in 40 mm dia and were measured using the 100 kv voltage to excite all the k alpha x-ray lines (Mohammad *et al*., 2016; Raju *et al*., 2016; Raju, 2019).

3. Results

The Kushta Gaodanti prepared by three different samples were analyzed for organoleptic properties, preliminary tests and physicochemical study. Moreover, elements and heavy metals were also estimated in all the three samples of Kushta Gaodanti.

Organoleptic properties: Appearance of all the three samples KGF1, KGF2 and KGF3 was found to be lusterless. Colour was observed to be dark grey in KGF1 and greyish white in KGF2 and KGF3. Smell KGF1, KGF2 and KGF3 was found odourless, and all three samples KGF1, KGF2 and KGF3 found to be salty in taste. Form of KGF1, KGF2 and KGF3 was found as fine powder.

Physicochemical parameters were analyzed in Kushta Gaodanti prepared by classical methods and the data presented in Table 1.

pH value: pH value of all the three Kushta is acidic. It is mentioned that most of the Kushtajat are alkaline and some are weakly acidic. pH of drug is also an important parameter to assess the quality of drug. The drugs that are weak acids, would be better absorbed from the stomach then from the upper intestine. Bulk density, total ash, loss of weight on drying at 105 °C, *etc.*, also authenticates the recommendation of Unani physicians to preserve the Kushta properties and establishing standards.

Table 1: Physicochemical parameters of Kushta Gaodanti

S.no	Parameter	Sample 1 KGF1	Sample 2 KGF2	Sample 3 KGF3
1.	Appearance	lusterless	lusterless	lusterless
2.	Colour	dark grey	greyish white	greyish white
3.	Smell	odourless	odourless	odourless
4.	pH	acidic	acidic	acidic
5.	Particle Size	<10µm	<10µm	<10µm
6.	Bulk density	0.95	0.97	0.87
7.	Total Ash (%w/w)	98.9924	99.8675	95.2413
8.	Acid Insoluble Ash (%w/w)	79.0452	73.2684	73.5726
9.	Loss of Wt. on drying at 105 °C (%w/w)	0.48122	0.4956	0.4901
10.	pH Value at 1% aq. solution	4.01	4.17	4.23
11.	Distilled water test	Sinks in water	Sinks in water	Sinks in water
12.	Still water test	positive	partially positive	partially positive
13.	Wall stick test	positive	partially positive	partially positive
14.	Floating test	positive	positive	positive
15.	smoothness	positive	positive	positive

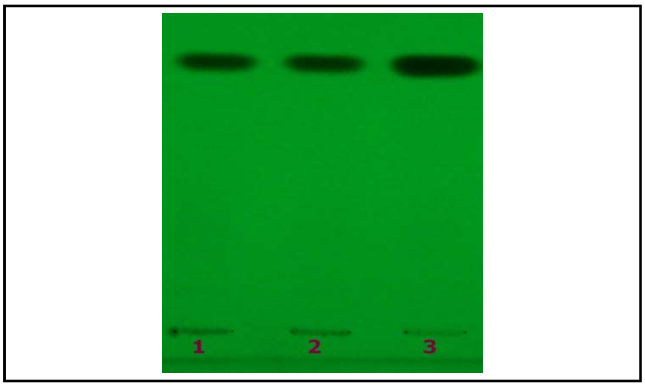


Figure 3: TLC plate of chloroform extract of Kushta Gaodanti. Three different samples developed with 100% hexane and detection at UV λ=254 nm wavelength (Track 1: KGF1; Track 2: KGF2; Track 3: KGF3).

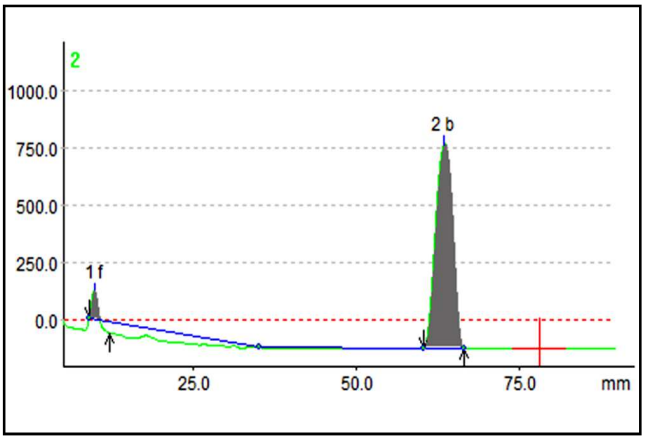


Figure 4: Densitogram of chloroform extract of Kushta Gaodanti at UV λ=254 nm.

HPTLC analysis

HPTLC fingerprint of chloroform extract of Kushta Gaodanti prepared by three different samples, were carried out and TLC plate was developed with 100 % hexane as mobile phase (Figure 3).

XRD-analysis

The non-destructive analytical technique using EDXRF is carried out using an in-built "Auto quant" program developed by (M/s Panlytical^{R}) using matrix match standards around 100 wt% element concentration of elements measured. The elemental abundance on an average in three samples consists of Calcium (Ca) ~12.33 wt%, Zinc,(Zn) 17.66 wt% with only exception to KNG-3 sample consisting of heavy metals like Arsenic with (As) 1 ppm and Lead (Pb) around 14 ppm. The SO_3 is a chemical compound of sulphur and trioxide, sulfite with sulphur and oxygen with 2-charge. On average, SO_3 present around ~62.33 wt%. To ascertain the enrichment of the elemental abundance's measured by EDXRF, we also corroborated the studies using XRD to understand the mineral species responsible (Figures 5-7).

4. Discussion

Preliminary tests: The Khusta Gaodanti samples prepared were analyzed for metallic luster and found to be negative in all the three samples KGF1, KGF2 and KGF3. The prepared Kushta was lusterless, which was in accordance to good quality of Kushta mentioned by Unani scholars. It is mentioned that the processing by which kushtajat is prepared leads to loss of luster. Finger thumb test was found to be positive in all the three samples KGF1, KGF2 and KGF3. Fineness test: The fineness and smoothness of Kushta indicates that the Kushta as deposited into crease and lines on ventral aspects of fingers, which confirms its smoothness and fineness, showing the particle size of Kushta was very small and also found below 10 nm with particle size analyzer. Stillwater test and Wall stick test was found to be positive in KGF1, KGF2 and partially positive in KGF3. Floating test was found to be positive in KGF1, KGF2, and in KGF3. Floating test findings was consistent with classical literature that an ideal kushta floats over the surface of water as floating on the surface of liquid. The Kushta particles though denser than water its size is very small and unable to overcome the force of surface tension of water, thus it floats on the surface of the water (Table 1).

Physicochemical parameters were analyzed in Kushta Gaodanti prepared by classical methods results are described as the particle size was determined using a laser scattering particle size analyzer (Malvern ZetaSizer ZEN3600, UK). The particle size was found to be less than 10 nm in all KGF1, KGF2, and KGF3. pH of 1 % aqueous solution was found to be 4.01 in KGF1, 4.17 in KGF2, and 4.23 in KGF3. Bulk density: The mean value of bulk density of all the three samples was found to be 0.95 in KGF1, 0.97 in KGF2, and 0.87 in KGF3. Total ash: The mean value of total ash of all the three samples

found to be 98.9924% w/w in KGF1, 99.8675% w/w in KGF2, and 95.2413% w/w in KGF3. The mean percentage of loss of weight on drying at 105°C was found to be 0.48122% w/w in KGF1, 0.4956% w/w in KGF2 and 0.4901% w/w in KGF3. Acid insoluble ash was found to be 79.0452% w/w in KGF1, 73.2684% w/w in KGF2 and 73.5726% w/w in KGF3. (Table 1)

HPTLC analysis

HPTLC fingerprint of chloroform extract of Kushta Gaodanti prepared by three different samples were carried out and TLC plate was developed with 100% hexane as mobile phase (Figure 3) and detected using the UV visible chamber which clearly showed a spot in UV 254 nm in the densitogram (Figure 4 and Table 2). The corresponding Rf value for the spot obtained in the TLC plate for the Kushta Gaodanti and the spot detected under UV wavelength of λ=254 nm at Rf value 0.90 (black). This helps in identification of Khusta under the same experimental conditions with single spot at Rf value of 0.90

Table 2: Peak list of chloroform extract of Kushta Gaodanti at UV λ=254 nm

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	9.8	118.01	4.24	122.68	0.01
2	63.6	2667.17	95.76	886.68	0.90

XRD-analysis

In the Kushta Gaodanti sample, *i.e.*, KNG -1, the calcium enrichment is due to calcium sulphate identified at 2theta 41 degree and a sharp peak of 2ctheta at 26 degrees indicative the presence of lead thallium. In KNG 2, the Nd_3NiGaS_7 (Nickel Gallium Neodymium Sulfide) is identified at 25.5 2theta angle, minor peaks of lead (Pb), ZrO, TiO0.577WO0.316 (Titanium-tungsten Zirconium), $V_2Mn_2O_7$ (Vanadium, Manganese Oxide Ti_2Cu_3, *etc.,* are also picked using Bruker TOPAS program. In KNG-3, sharp peaks of calcium contents is due to presence of anhydrite ($CaS0_4$) and Oldhamite, *i.e.,* a CaS form. (Coupled TwoTheta/Theta Figures 5-7)

Metal analysis

Calcium analysis was carried out in KGF1, KGF2 and KGF3. The result was found to be 36.46%, 36.93% and 38.29%, respectively. The elemental percentage was found to be similar in all the three samples, but slightly higher in third sample, *i.e.,* KGF3.

Iron and magnesium analysis were also done in all the said three samples. The presence of iron was found to be 0.08%, 0.07% and 0.10%, while the magnesium presence was found to be 0.04%, 0.08% and 0.20%. Here, the different values amongst the various preparation methods of iron and magnesium are insignificant. However, the difference in third sample may be due to the use of Asgand and Gheekwar in its whole form. Thus, the results of Kushtae Gaodanti in third method of preparation suggest high yield in comparison to other methods of preparation.

Heavy metal analysis

The analysis of heavy metals as per the methods described in WHO guidelines. Anonymous (1998) is carried out in the above mentioned three samples of Kushtae Gaodanti. The results of heavy metals determination were within the permissible limits as given in Table 3. The value of cadmium, mercury lead and arsenic was below detectable amount in KGF1 and KGF2. Whereas in KGF3, the arsenic was found to be 1 ppm which is a permissible limit. The lead (Pb) value was found to be 14 ppm which is exceeding the permissible limit (10 ppm). Here, the probable cause of higher values in KGF3 may be due to the preparation method of third sample adopted where the raw *asgand* powder and *gheekwar* were used in their whole form avoiding pellatization process. Due to which, the heat transformation could have not been equally distributed. XRD-analysis showed the presence of calcium and silicate in all the three samples of Khusta-e-Gaodanti, prepared by classical method. Traces of copper (Cu), Zinc (Zn), Chlorine (Cl) are present. All the above metals are useful to human body. But heavy metals like Arsenic (As), Strontium (Sr) and lead (Pb) are also present in the third sample of khusta-e-Gaodanti.

Table 3: Heavy metals and elemental analysis by EDXRF in Kushta gaodanti

S. No.	Result	Units	KNG-1	KNG-2	KNG-3
1.	CaO	%	36.46	36.93	38.29
2.	K_2O	%	0.04	0.08	0.20
3.	Fe_2O_3	%	0.08	0.07	0.10
4.	SiO_2	%	0.03	0.00	0.00
5.	SO_3	%	63.24	62.78	61.14
6.	Cr_2O_3	%	0.00	0.00	0.10
7.	Cl^-	%	0.11	0.09	0.15
8.	Cu	ppm	12.00	15.00	10.00
9.	Zn	ppm	13.00	18.00	22.00
10.	Sr	ppm	295.00	333.00	282.00
11.	As	ppm	0.00	0.00	1.00
12.	Hg	ppm	0.00	0.00	0.00
13.	Cd	ppm	0.00	0.00	0.00
14.	Pb	ppm	0.00	0.00	10.00

Physicochemical parameters were analyzed in Kushta Gaodanti prepared b Gaodanti prepared in decoction of Asgand also have no traces of arsenic and lead.

Gaodanti prepared in both *Aloe vera* and asgand powder has traces of arsenic and lead as shown in Table 3. Hence, this formula is not advisable for treatment as it contains heavy metals.

Though strontium is present in high amount (280-333 ppm), as strontium is absorbed by the body in similar manner to that of calcium, is not hazardous to health.

As no standard physicochemical profile of Kushtae Gaoanti is reported till date, the current data may be considered as standard for future studies. The analytical result showed the presence of calcium and copper in KGF1, KGF2 and KGF3, whereas traces of arsenic and lead are present in KGF3.

The studies done by Dubey *et al.* (2008); Takkar *et al.* (2017) and Khan *et al.* (2012) conform the calcium presence in Kushtae Gaodanti to 29.32%, 42.30% and 29.30%, respectively. The study done by Central Council for Research in Unani Medicine, New Delhi, India also suggests the presence of calcium in Kushtae Gaodanti with 32-38 mg/gm amount being closer to reported results.

Hence, the present study was aimed to prepare Kushtae Gaodanti by triturating in two different media with different weights of cow dung cakes (KGF1 and KGF2), one method prepared by without trituration and pellatization (KGF3). The data of the present study suggest that the physcicochemical characteristics of KGF1 and KGF2 were similar, whereas KGF3 differs in heavy metals like arsenic and lead values. Hence, the study validates trituration and pellatization plays an important role in the preparation of Kushtajat.

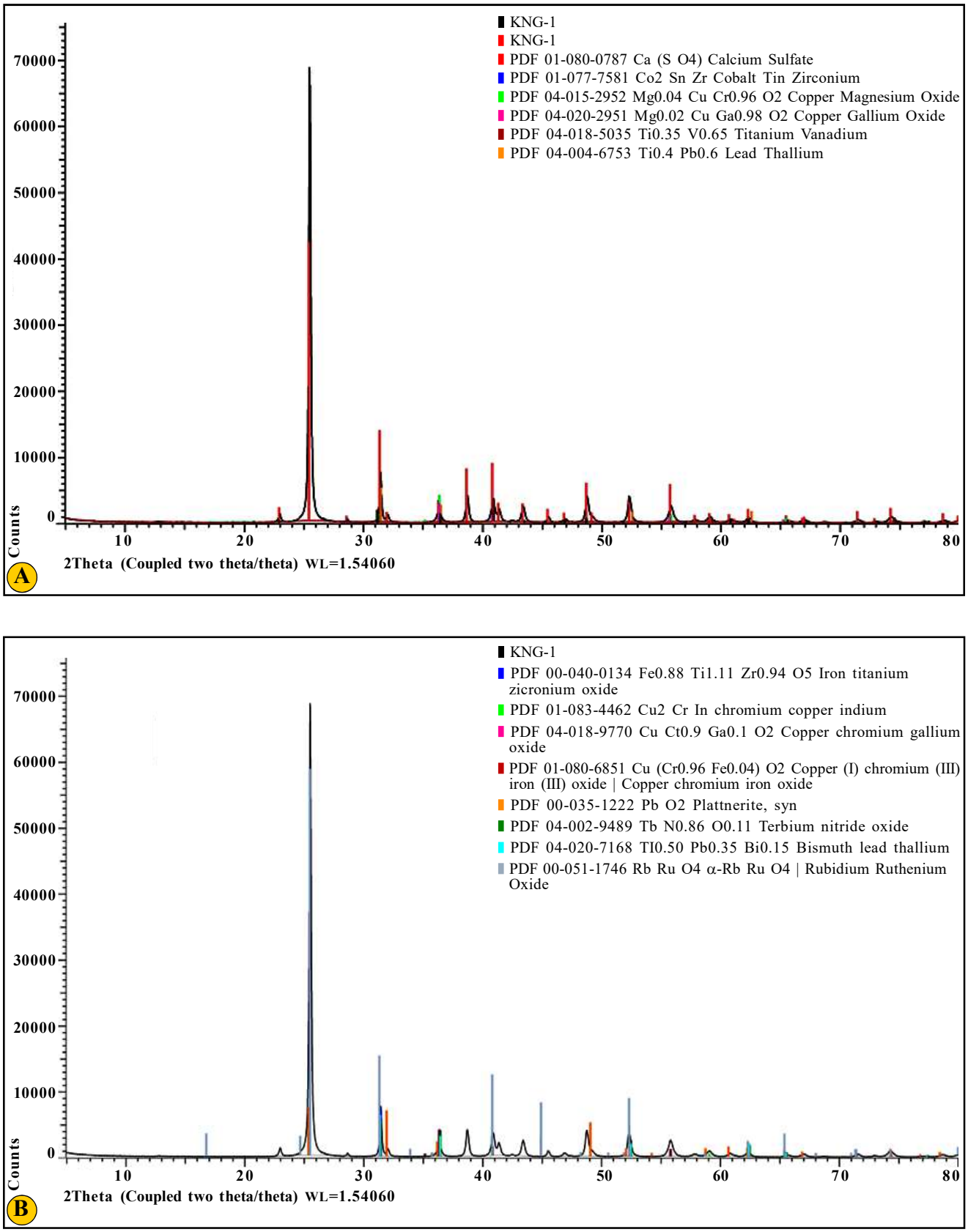


Figure 5: 2Theta_(Coupled two theta/theta) for KGF1(A-B).

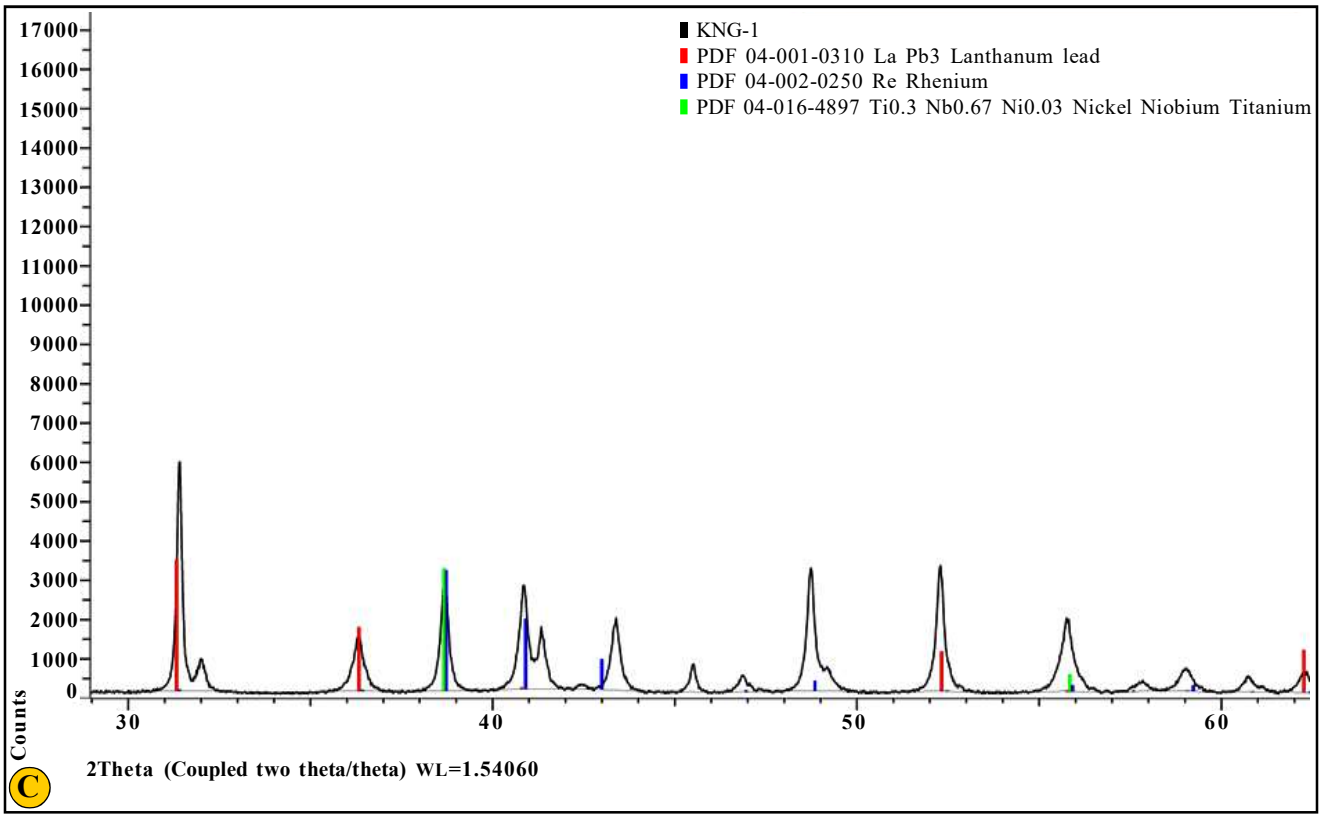


Figure 5: 2Theta_(Coupled two theta/theta) for KGF1(C).

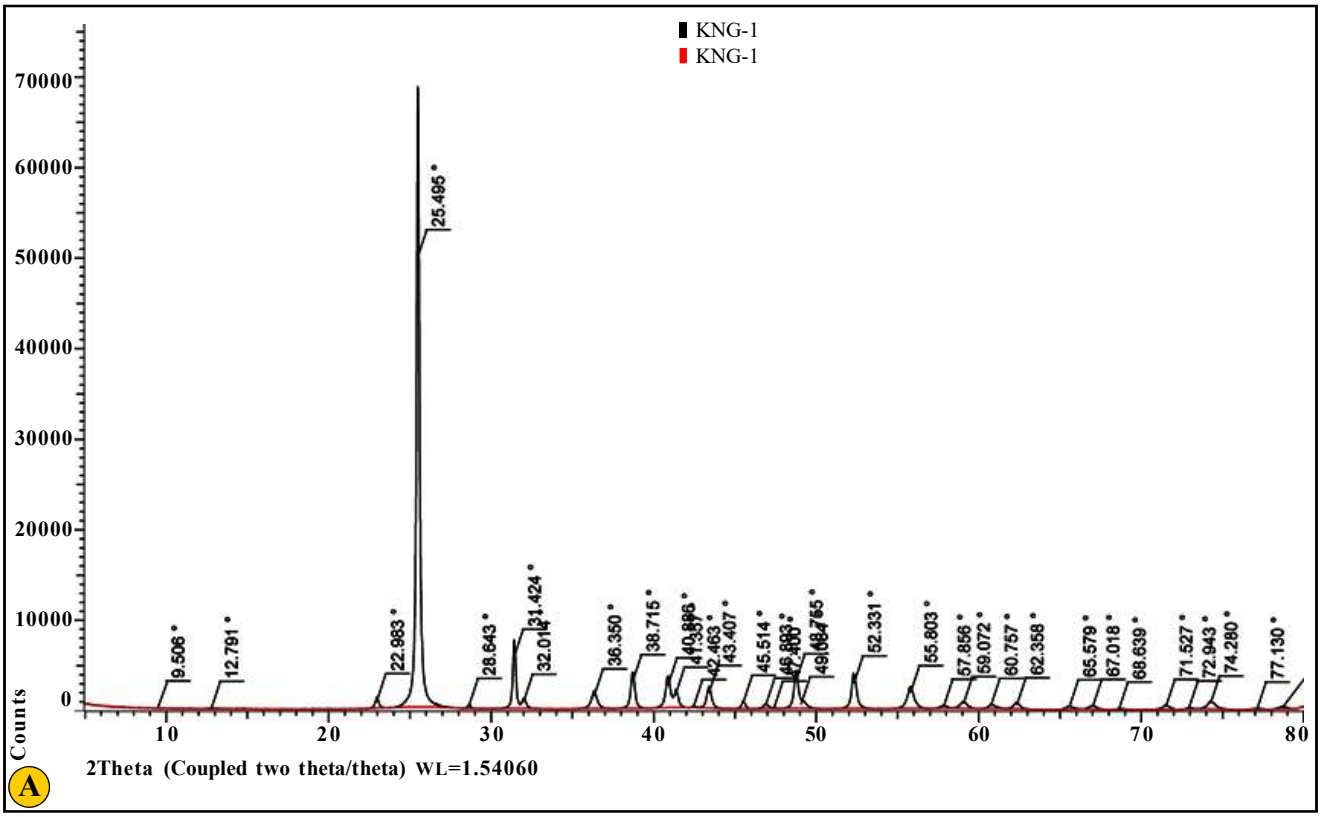
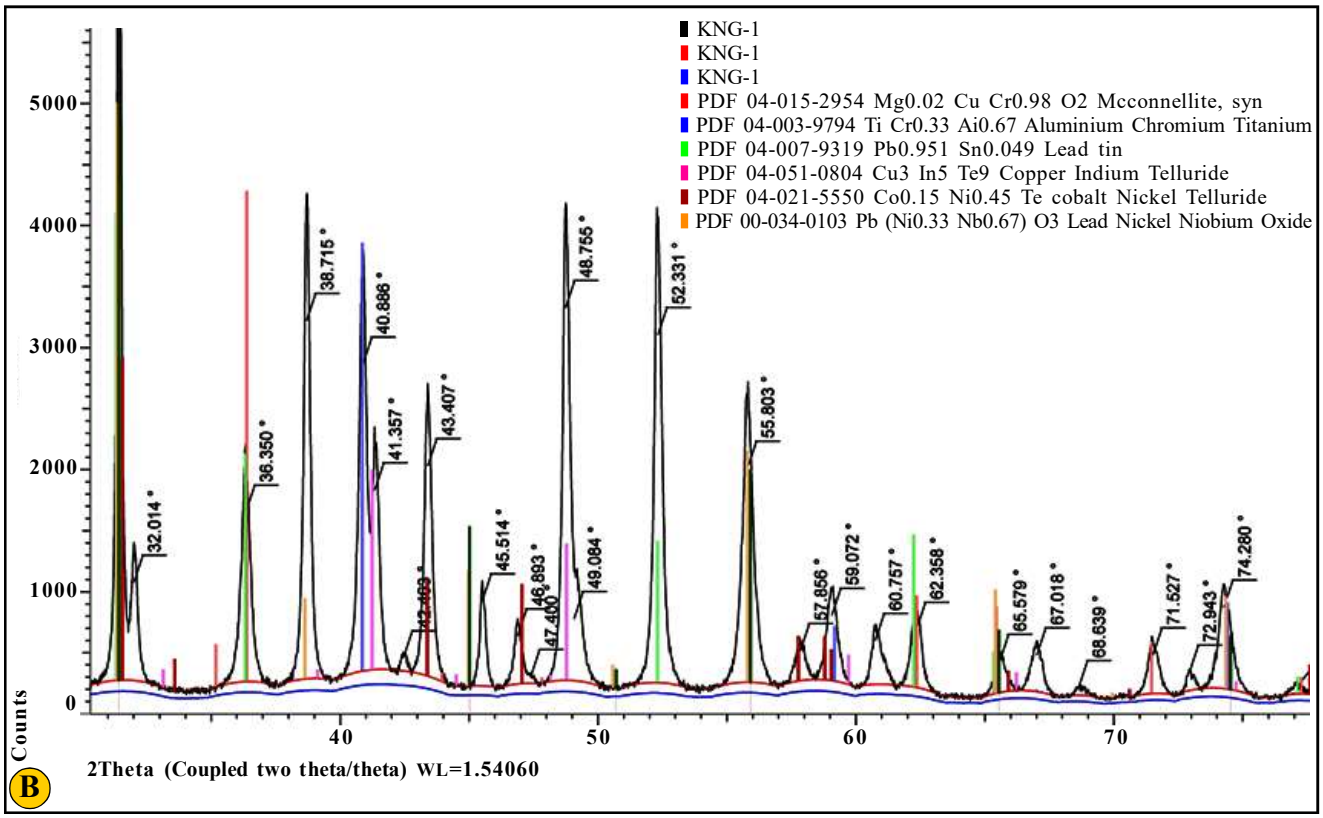


Figure 6: 2Theta_(Coupled two theta/theta) for KGF 1&2 (A).



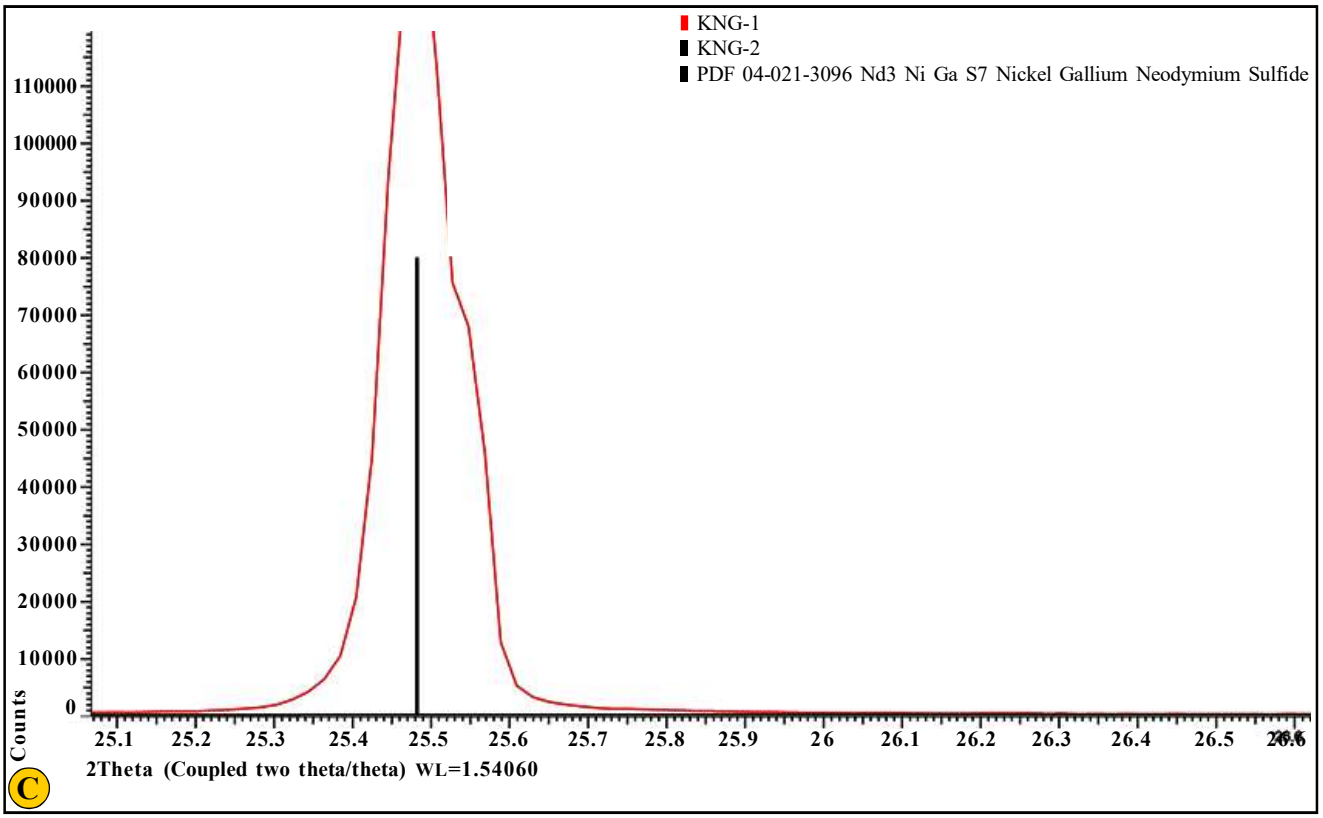
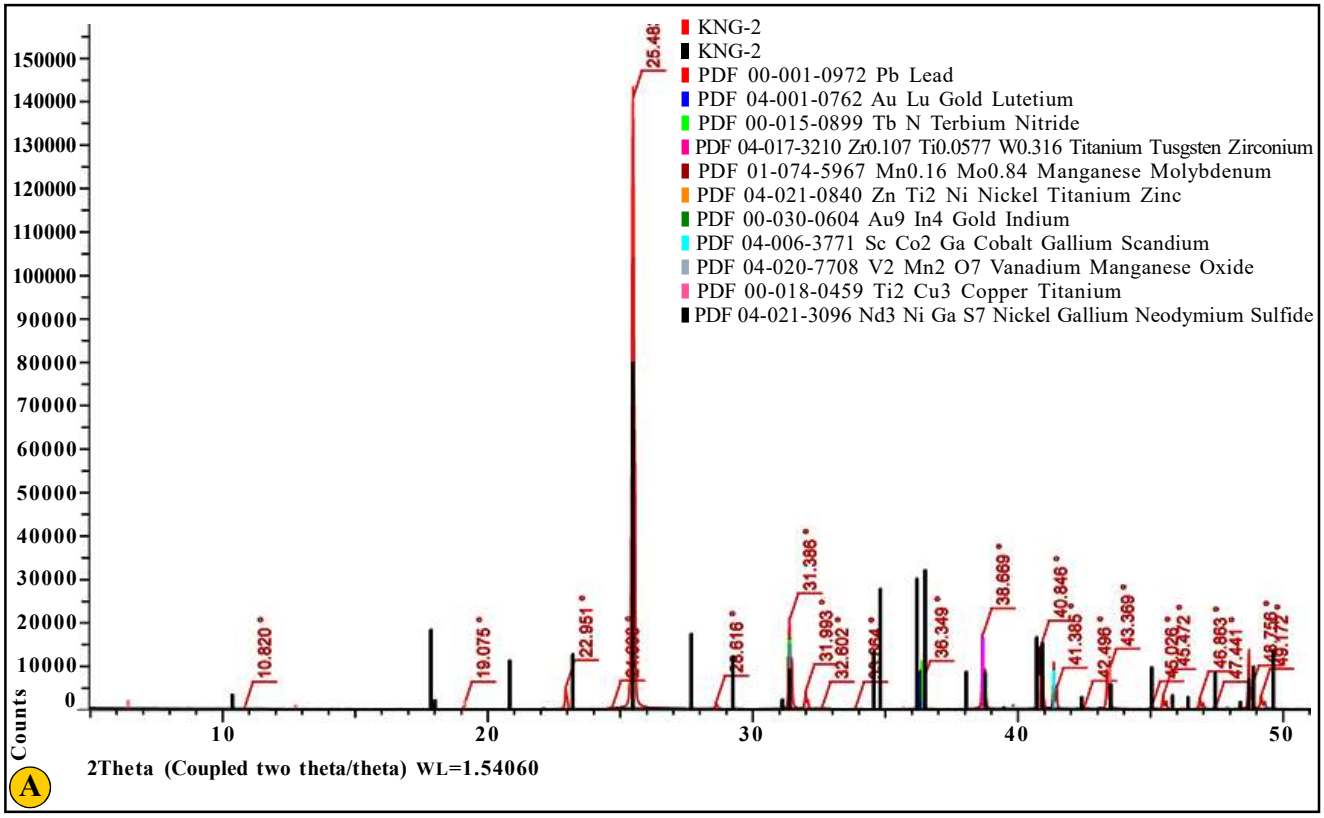


Figure 6: 2Theta_(Coupled two theta/theta) for KGF 1&2 (B-C).



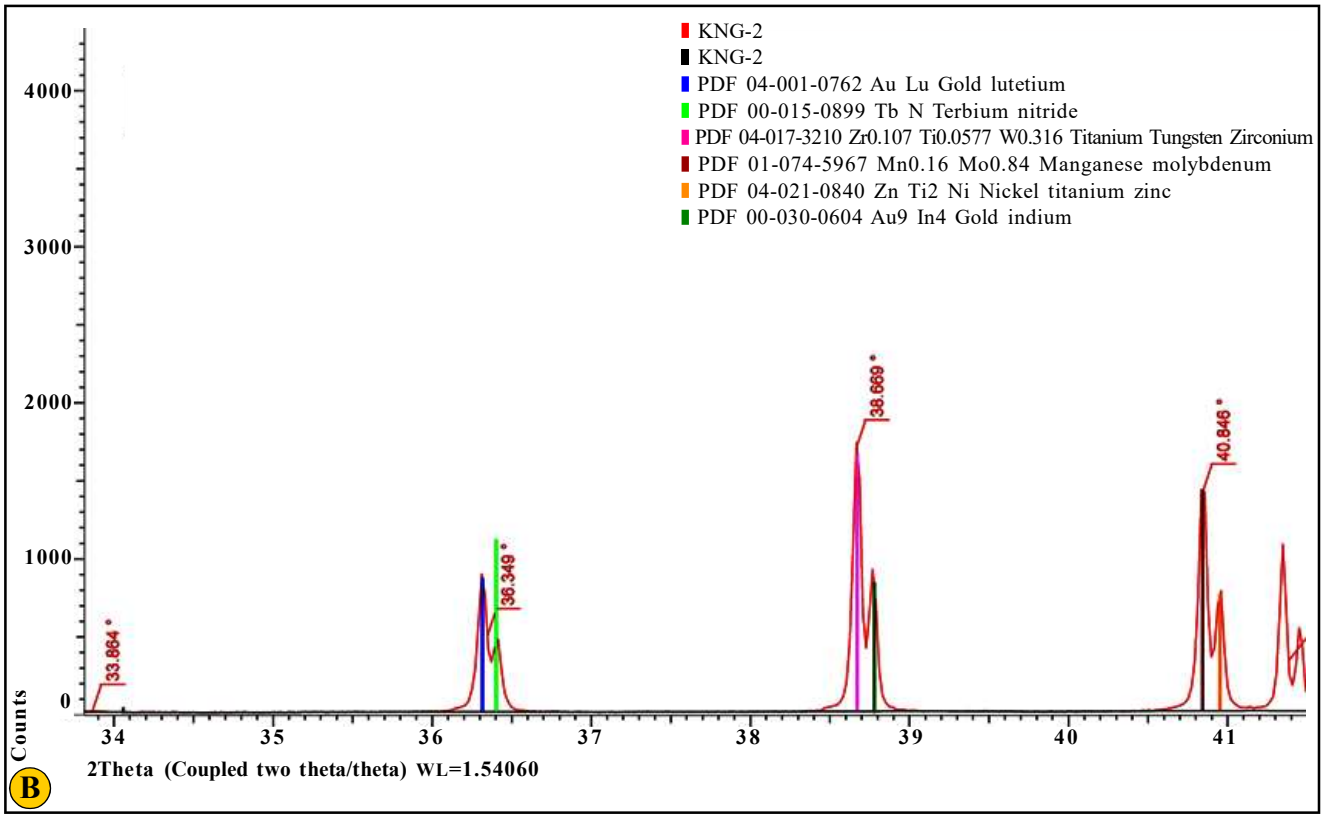


Figure 7: 2Theta_(Coupled two theta/theta) for KGF 2&3 (A-B).

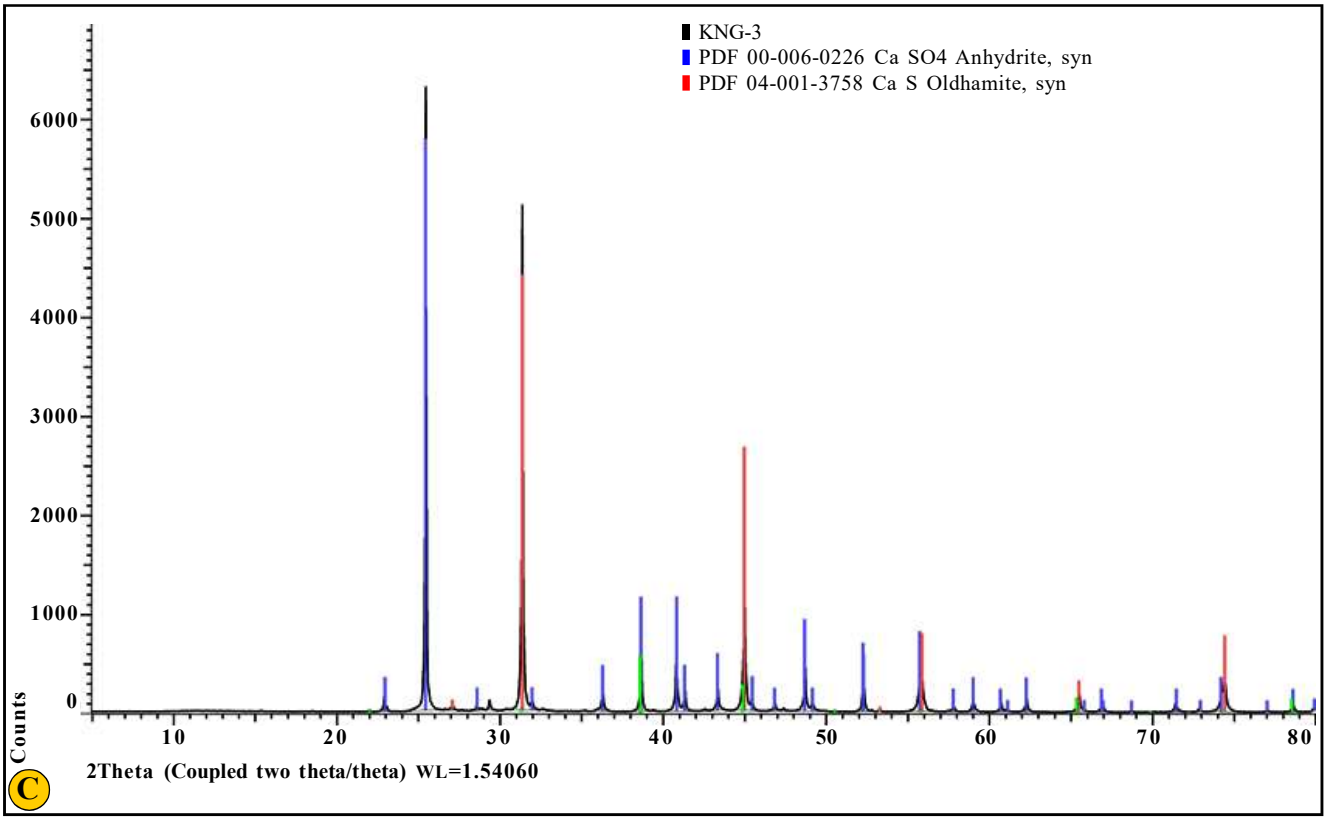


Figure 7: 2Theta_(Coupled two theta/theta) for KGF 2&3 (C).

5. Conclusion

The Unani formulation, Kushtae Gaodanti was subjected to various parameters to develop the standard operating procedures such as physicochemical parameters which helps as a standard along with the other parameters like HPTLC analysis, metal analysis, XRD studies. Safety evaluation of drug such as heavy metal analysis, contamination analysis suggested that the drugs are safe and can be used in further studies such as pharmacological study, clinical study, *etc*. Modern technique of HPTLC fingerprint analysis was employed and it is an important study in standardization and facilitate in separation of compounds present through isolation for further studies. Consequently the Kushtae Gaodanti drug was brought up in its quality to set as a reference standard. The present study serves in the quality assurance of Kushtae Gaodanti in the Unani System of Medicine with standardized parameters. The development of AYUSH medicines or traditional or Unani system of medicines with respective to safety, efficacy and quality will help not only to preserve the traditional heritage but also in the rationalize use of natural herbal products in the healthcare. Further, studies in the direction to evaluate the therapeutic efficacy of KGF1 and KGF2 by conducting animal trials may be conducted and also to confirm the detoxification related change, pharmacological effects should be evaluated *in vivo* studies on scientific parameters.

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Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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