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SYNTHESIS AND CHEMICAL CHARACTERIZATION OF AYABIRUNGARAJAKARPAM-A HERBO-METALLIC SIDDHADRUG

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ABSTRACT

Background: This experimental research study dealt with the preparation and characterization of *Ayabirungaraja karpam*(ABK) which underwent various levels of purities to make it free from untoward effect. The sophisticated analysis of instruments were an important criteria in studying the surface morphology, the chemical bond present which indirectly correlates with the active form and also an important feature of the standardization of the drug. **Objective:** To prepare the drug in *shastrial* method and to focus with specific aim to establish chemical characterization studies of ABK with sophisticated instruments. **Methods:** The preparation and purification of ABK involves heating and drenching, impregnation and solar heating. The chemical characterization was carried out with EDS, FE-SEM, FTIR, XRD, XPS, XRF, TG-DTA, AAS. **Results:** SEM analysis showed that the roughness morphology seen on the surface of ABK. EDS shows the presence of iron, silicon, aluminium, magnesium, sodium, carbon, oxygen etc. XRD analysis showed the indexed pattern of ABK sample which indicating the formation of Fe_2O_3, XRF analysis showed the presence of mineral like Fe, Si, Al, Mn, K, Cl, Cu and Zn. On the surface of the drug by XPS supports the confirmation of coating of organic molecules. TG-DTA analysis showed the view of change in the weight of the substance with respect to the temperature over a period of intervals. FTIR analysis showed the peak value which are functional group present in ABK. AAS showed the heavy metals like mercury, cadmium, arsenic, lead were present within the permissible limit. **Conclusion:** The toxic heavy metal free ABK pharmacologically act as well absorbent, adsorbent and biocompatible drug.

KEYWORDS: Standardization, biocompatible, Adsorbent, heavy metals, Absorbent.

INTRODUCTION

The use of heavy metals and minerals is a integral part of siddha Medicine which is known as "*Rasavathakalai"* or iatrogenic chemistry of siddhars. The formulation of siddha drugs are prepared by herbal, metal, mineral and animal origin. The pharmaceutical process of herbo-metallic and herbominral siddha drugs should undergo the steps in respect to the specific methodology viz Purification or potentiating(*Suththi*), Impregnation or levigation (*Bhavanam*), Incineration or calcinations (*Maranam*) The detoxification of raw materials and process of preparation of herbomineral drug undergo several traditional steps while drug become more potent.[1]

Purification is the process, by which detoxification of the toxic materials may occur. Some materials are used readily as therapeutic agent once the purification finished since the media may have some active principle which is body friendly. There are fifteen steps involved in several methods of purification.eg; immersing process and flaming, drenching in liquid media which make smoothness, reduction in particle size, fragile and rough surface of the metal and minerals. The particle size reduction help in increase absorption and smooth flat surface leads to non irritability, thus these physicochemical changes increase the bioavailability and biological potency to the processed material. Impregnation is the wet grinding process while materials are grind with specific liquid media for stipulated time to bring minute particle by absorbing the plant juices in contact with the material, thus, then transferred the processed material into organometallic or organo-mineral compounds which are compatible to the human system.[2]

Calcinations or incineration process converts the purified metals and minerals into *parpam* and *chenduram*. It is accountable for numerous changes such as formation of desirable compounds, conjugation of the trace elements, fulfil the demand of trace elements in the body, size reduction of particle which favours the easy and quick absorption and elimination of toxic and other unwanted material.[3] *Ayabirungarajakarpam*(ABK) is well known drug among Siddha medical practitioners which is used commonly for anemia without any proper scientific study. Therefore, this paper dealt with preclinical validation through scientific chemical characterization to claim the ABK as safe and effective drug thus, in the broader sense standardization of ABK involves with spectroscope, thermo gravimetric, Microscope

studies and X-ray technique in this research study to expedite the surface morphology and nano particle size.

MATERIALS AND METHODS

Preparation of ABK

Procurement and Authentication

The main ingredients raw ore iron (*Ayam)* and raw cast iron (*mandooram*) were purchased from Trichy local market and obtained authentication from Dr.KKadirvelu, retired Professor of Geology, V. O. Chidambaram College, Tuticorin. *Wedeliachinensis* (Osbeck) Merr was collected from local herbal garden, Thanjavur and *Citrus limon* L fruit was purchased from local market, Thanjavur and authenticated with Voucher specimen No CARISM 109 and Voucher specimen No CARISM 110 respectively by Dr Ravichandran, Asst. Professor Botany, CARISM, Sastra, University, Thanjavur.

Purification process

The purifying agents are *annakhadi*, is a six months old Fermented acidic rice water sesame oil, cow urine and *Kollukudineer* (Standard 4:1 ratio the process was repeated three times for heating, quenching and washing while every time fresh *annakhadi* and water were used. The same purification steps were carried out with300mL sesame oil, 300mL Cow urine and 300mL *kollukudineer* respectively. Altogether twelve times put in the four types of different liquids to obtain purified form of *Ayathool*. The next ingredient of *Mandooram* 300g was weighed which was grounded well and put in a pan and heated up to red hot, then added 4 times weight of Tamarind leaves, 8 times of water and boiled for 3h. Once *manduram* cooled which was rubbed with boiled macerated tamarind leaves while washing. Then allowed to dry and residues were removed. The next step of purification process was heating this manduram with 8 times weight of cow urine, then washed and allowed for drying to obtain the purified mandurm.[4]- [6]

Pharmaceutical process

The preparation technique was mainly drenching metals in herbal juices and drying under solar treatment. Purified Aya powder 140g and purified Mandura powder 210g were mixed well and they were drenched in *Wedelia chinensis* juice in the volume of 1.3L correspond to this ratio and the mixture was kept under sunlight heat (*Sooryapudam*) for drying of *Wedelia chinensis* juice. Once it was dried up completely, the lime juice (*Citrus limon*) 1.3L of volume was added and drenched for complete drying under solar heat. Again, the drying

process was repeated by adding only *Wedelia chinensis* juice in the half of the volume of 1.3Luntil become waxy consistency. During the drying, the product was regularly mixed with spatula. Then the waxy end product was transferred to herbal cup (*thonnai*) made by dry leaves of *Ficus bengalensis,* for complete drying under solar heat. Finally the dried product was ground it as fine powder to obtain *Ayabirungarajakarpam*.[7]

Standardization by sophisticated instrumental analysis

X Ray Diffractrometer-XRD

In order to understand the crystalline behaviour of the ABK samples powder x-Ray diffractometer (XRD, D8 Focus, Bruker, Germany) instrument was used. The 1g samples were tightly packed on a polymer pan and exposed to the x-rays produced from Cu target. The applied voltage and current were maintained as 40 kV and 40 mA. The scan was performed from 20° to 80° with the scan speed of 0.01°/sec. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The ABK fine powder was pressed into the sample holder, which have a smooth surface and hold the sample at angle 45^0. High voltage generated and bombarded on a copper source, the X-ray produced from copper was directed through the sample and collected the signal by scintillation counter. *Annabethi chenduram* study method was adopted.[8]

X Ray fluorescence micro analyser- XRF

X-ray fluorescence analysis (XRF, S8 Tiger, Bruker, Germany) was done to know the elements present on the ABK samples. The samples were placed on a pan which was filled with two grams of boric acid (filler) and made as pellet. The high pressure of about 25 tonnes was applied to pelletize the aluminium cups with ABK sample. Similarly *Nagabhasma* characterization method was adopted.[9]

X ray Photo Electron Spectroscopy Analysis-XPS

X-ray photo electron spectroscopic (XPS, K-α**,** Thermo Scientific, USA) studies were performed to know the elemental composition along with its oxidation states. The ABK sample was pressed in the form of pellet and placed in the sample treatment chamber. The vacuum was developed in the order of 2×10^{-6}Torr before placing the sample in to the sample analyzing chamber to get vaccum of the order10^{-8}-10^{-9}Torr. The X-ray power supply was maintained at 15 Kv and 5 mA, respectively while scanning the ABK samples. Samagandhaga Kajali characterization method was adopted.[10]

Energy Dispersive X ray Analysis with Field Emission Scanning Electron Microscope - EDS with FE-SEM

The field emission scanning electron microscopy (FE-SEM, JSM 6701F, JEOL, Japan) was used to image the surface morphology of the ABK samples whereas energy dispersive x-ray analysis (EDS, Oxford, USA) was done to know the elemental presence on the surface of the ABK samples. Initially, the sample was deposited on a carbon adhesive tape which is attached on a metal stub. It was then coated using Au to make the surface more conducting and placed inside the instrument. The secondary electrons produced from the samples were captured using SEI detector while applying the voltage of 3 kV. The images were taken at different magnifications with various working distances in order to elucidate the surface of ABK sample. Study of *Sanguparpam* method was adopted.[11]

Fourier Transform Infra-Red spectroscopy. FT-IR

The functional groups present on the surface of the ABK samples were analysed using fourier transform infra-red spectroscopy (FT-IR, Spectrum 100, Perkin Elmer, USA). The samples were dried and mixed with KBr (1:50 ratio). It was then mixed thoroughly with the aid of agate mortar & pestle and made as transparent pellets. Then the pellets were put in the instrument and recorded the spectra between 500 and 4500 cm^{-1} wave number. *Kantacenturam* study method was adopted.[12]

Thermogravimetry - TG-DSC-DTA

Thermogravimetry analysis (TGA) (Q600, TA Instruments, USA) was carried out to understand the thermal behaviour of ABK sample. The test sample ABK was initially weighed (approximately between 2 and 5 mg) and put on the pan which is connected with weighing balance. It was then heated from room temperature to 1000 °C under reduced atmosphere (100 ml/min N_2 flow). The data were collected and plotted the percentage weight loss against temperature. *Lauha bhasma* characterization method was adopted.[13]

Atomic Absorption Spectrometer Analysis

The ABK 0.2g was taken in 100mL volumetric flask and about 4mL of HNO3 was added and solution was allowed to stand for few hours, then it was carefully heated over water bath till red fumes coming from the flask completely ceased. Flacked was allowed to cool at room temperature and about 4mL of perchloride acid was added and heated again over water bath to evaporate till a small portion which was filtered through no 42 filter paper. Atomic absorption spectroscopy (AAS) was used for detecting heavy metals in ABK. Atomic

absorption is the process that occurs when a ground state atom absorb energy in the form of light to the specific wavelength increased as the number of atoms of the selected elements in the light path increases. The relationship between the amount of light absorbed and the concentration of analytics present in known standard could be used to determine unknown sample concentration by measuring the amount of light, they absorbed. *Lauha bhasma* detection method was adopted.[14]

RESULTS AND DISCUSSION

The concept of using nano metal particle is prevailing since saint Bohar period for metallic preparation. Herbometallic drug play important role in Ayurveda and siddha therapeutics because of their qualities such as minute dose, good palatability and fast acting and also have anti-ageing quality, immune modulator and ability to target drug to the site(*Yogavahi* character). After the series of preparation process like potentiating or purification, impregnation and incineration of herbometallic drug become nontoxic, instant absorbable, adaptable and assimilable in the body.[15] There is evidence to show that old molecules are finding new applications through a better understanding of conventional knowledge and clinical observation. Therefore, the conventional knowledge, modern medicine, and biomedical sciences form a triangle platform to invent innovative newer, safer, cost effective and efficacious formulations.[16]

XRD analysis

X-ray techniques such as XRD, XRF and XPS have been used to know the crystal phase, elemental composition and oxidation state of the iron in ABK, respectively. Figure 1 shows the powder XRD patterns of ABK and its ingredients. As raw iron and manduram have been taken initially and purified to form ABK, the XRD of raw as well as purified samples have been analysed (Figure 1a, b, c & d). The raw iron and manduram [Figure 1(a)&(b)] show the iron oxide phases due to oxidation processes happed normally on the exposure of iron to the moisture under high temperature. In the process of purification, the raw materials were treated with organic materials such as sesame oil, cow urine, *Kollukudineer(Macrotylo mauniflorum seeds)*So, XRD patterns of purified iron and *manduram* (Figure1(c)&(d)) show the comparatively low intense peaks of iron oxide. This may be due to the removal of iron oxide from the surface of raw materials. Most of the organic materials or juices used here contain acids which may act as etching agent to clean the surface of raw materials. Figure 1(e) shows the pattern of lemon juice and *W.chinensis* extract treated raw iron and *manduram*

mixture. It is also found that there are no crystalline peaks of iron oxide found due to the introduction of acid-based extracts. The final product ABK was attained after drying it completely on *Ficus benghalensis* cup whose XRD pattern was showed in Figure 1(f.) The indexed XRD pattern of ABK sample well matched with the standard PDF card (JCPDS No. 86-2368), indicating the formation of Fe_2O_3. The drying process on *thonnai* (Ficusbengalensis) may help to oxidize completely to form Fe_2O_3. The similar analysis was performed in study of *lauha bhasma*.[17]

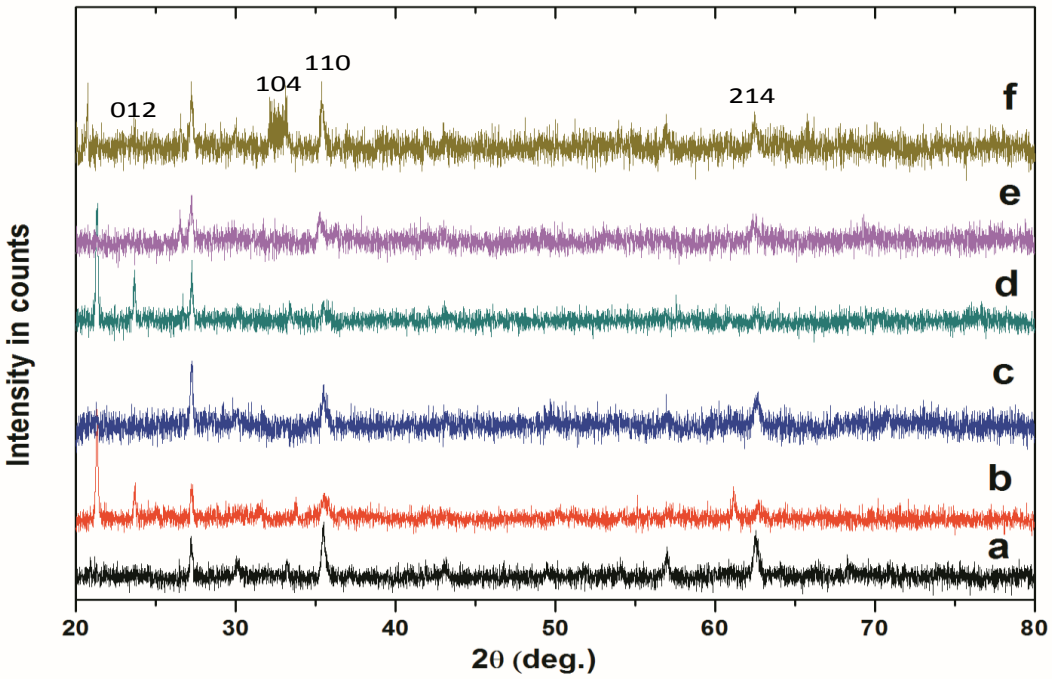

Fig. 1: Powder XRD patterns (1a) raw iron, (1b)r aw manduram, (1c) purified iron, (1d) purified manduram, (1e) purified iron-manduram mixed with lime juice and birungaraja extract and final (1f) ABK drug.

XRF analysis

The elemental composition of raw iron, raw *manduram*, purified iron, purified *manduram* and ABK have been studied using XRF and tabulated (Table 1) here. The results show that along with 91.88% of iron, 5.02% of Si is found in the raw iron whereas it is calculated as 94.99% iron and 2.19% Si in the purified form. The increase in the iron content confirms the removal of some of oxides from the raw materials. Also, it is interesting to know there is decrease in the percentage of Si in the purified form. This may be due to the addition of organic materials during purification processes. The same trend is noticed in the *manduram* sample which shows 75.76% Fe & 11.57% Si in the raw material and 78.75% Fe and 9.71%

Si in the purified form of *manduram*. In the final form of drug ABK, the iron content was found to be 77.23%. But there is notable amount of Si and Ca found in the sample which is almost 5.46 and 5.20%, respectively. Similarly, XRF analysis was done previously in *Lauhu bhasm.*[18]

The final drying process under sunlight on *thonnai*, (Ficusbenhalensis leaf herbal cup) may influenced on the inclusion of various other minerals along with iron. *Ficus benhalensis* leaf contain high level of water soluble antioxidant, polyphenols and flavonoid.[19] The elements present in the ABK formulation will play significant role in the therapeutic effect of the drug because iron makes haemoglobin and myoglobin which are respectively carries oxygen in the blood and hold oxygen in the muscle. Calcium assists in releasing hormone, helps blood vessels and carries blood to all parts of the body. Magnesium helps in regulate heartbeat. Zinc assists in growth & development and immune function. Sodium maintains blood volume, blood pressure and helps muscle nerves work properly. Potassium and chlorine are the essential elements to maintain fluid balance and acid base balance. Phosphorus is a part of every cell membrane and also strengthens the Haemoglobin formation. Sulphur is making protein and helping to remove toxins. Copper enhance the antioxidant reaction. Manganese helps to produce energy and antioxidant. These beneficial elements enhance the therapeutic effect by increasing the bioavailability of the drug.

Table 1: Elemental composition of raw Fe, raw manduram, purified Fe, purified manduram and ABK, analysed using XRF.

S.N	Raw iron	%	Purified iron	%	Raw manduram	%	Purified manduram	%	ABK	%
1	Fe	91.88	Fe	94.99	Fe	75.76	Fe	78.75	Fe	77.23
2	Si	5.03	Si	2.19	Si	11.57	Si	9.71	Si	5.46
3	Al	0.95	Al	0.36	Al	3.85	Al	3.29	Al	1.01
4	Mn	0.52	Mn	-	Mn	0.29	Mn	0.32	Mn	0.37
5	K	0.10	K	0.36	K	0.87	K	0.95	K	3.08
6	Mg	0.20	Mg	0.25	Mg	0.46	Mg	0.41	Mg	0.79
7	Ca	0.30	Ca	0.24	Ca	-	Ca	-	Ca	-
8	S	0.19	S	0.13	S	-	S	0.14	S	0.65
9	Cr	0.11	Cr	0.10	Cr	-	Cr	0.03	Cr	0.08
10	Ti	0.08	Ti	0.05	Ti	0.17	Ti	0.17	Ti	0.08
11	Na	0.42	Na	-	Na	-	Na	0.13	Na	-
12	Cl	0.05	Cl	0.22	Cl	0.19	Cl	0.08	Cl	3.60
13	Cu	0.05	Cu	0.05	Cu	0.01	Cu	-	Cu	0.04
14	P	0.05	P	0.08	P	0.14	P	0.15	P	0.36
15	Ba	0.02	Ba	-	Ba	0.03	Ba	-	Ba	-

16	Ni	0.02	Ni	-	Ni	-	Ni	-	Ni	0.03
17	Zn	0.02	Zn	0.06	Zn	0.02	Zn	0.04	Zn	0.07
18	Zr	61PPM	Zr	72PPM	Zr	71PPM	Zr	0.03	Zr	-
19	V	-	V	57PPM	V	0.06	V	0.06	V	0.03
20	Ce	-	Ce	-	Ce	72PPM	Ce	-	Ce	-
21	Mo	-	Mo	-	Mo	0.01	Mo	-	Mo	-
22	Sr	-	Sr	-	Sr	0.03	Sr	0.05	Sr	-
23	Pb	-	Pb	-	Pb	-	Pb	-	Pb	0.14

XPS analysis

The XPS analysis provides valuable information for the surface state of the ABK drug sample. Fig. 2. shows And confirming the presence of iron in ferric form, calcium, carbon and oxygen, which are the building blocks of the organic materials, on the surface of the drug by XPS supports the confirmation of coating of organic molecules on the surface of the metallic compounds. In addition to this, the EDS Figure 3 and XRF analysis showed Na, Mg, Al and Si which are not seen under XPS surface spectra. This may be due to presence of their low amount or lesser than the detectable limit by XPS. The analysis was already noted in previous studies in Fe^{2+} & Fe^{3+} irons in oxide materials.[20]

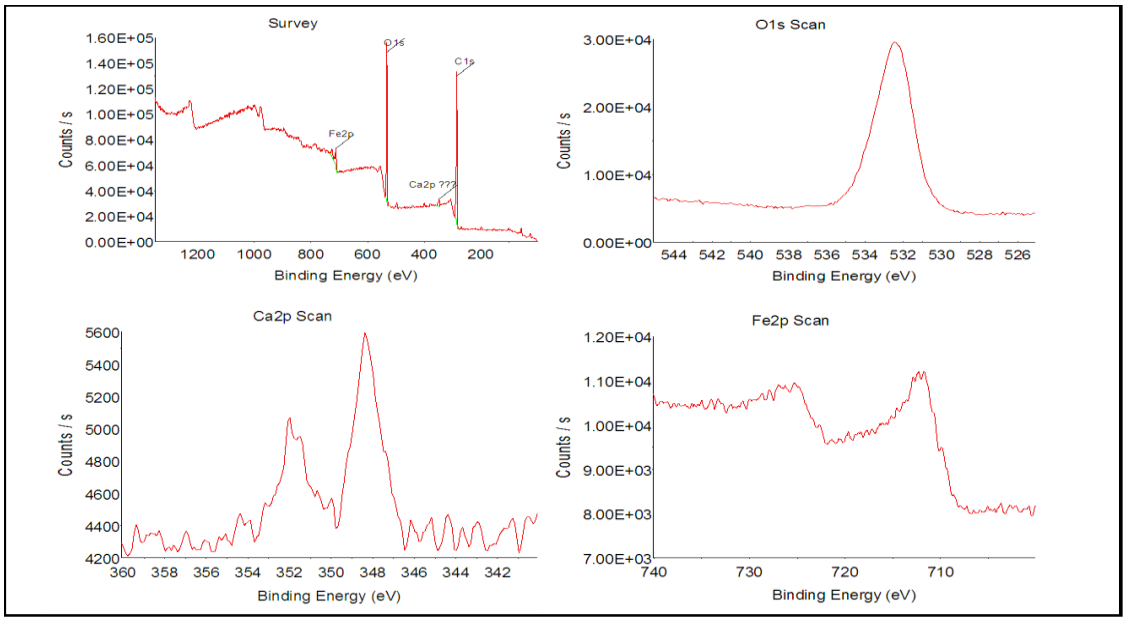

Fig. 2: XPS analysis of ABK drug.

Microscopic studies with SEM

Plate 1 shows the FE-SEM images of ABK drug. One can view the aggregated morphology of ABK in the Plate (1a) whereas fine nanoparticles were found in the high magnified FE-SEM image Plate (1b). The roughness morphology seen on the surface of ABK drug may be

due to the aggregation of nanoparticles during various processes on drug preparation. The processes include the treatment of lime juice and *birungaraja(manjalkarisali)* extract which may act as coordinating legend with iron present in raw ayam and raw mandooram. The formation of metal-ligand complexes increases the solubility of the drug which tends to aggregate to form the microstructures or nanostructures. ABK particle size is 100nm and the particle was stabilized adding lime juice contain citrate in the preparation of ABK, have the ability to migrate into cells and body compartments is due to its unique small size EDS (Fig. 3.) shows the presence of iron, silicon, aluminium, magnesium, sodium, carbon, oxygen etc.

The similar past research study was noted in particle size estimation and elemental analysis.[21] The iron based nano medicine on magnetite Fe_3O_4 and maghematite 8 Fe_20_3 are popular and some products based on these materials have been approved by the US FDA for the clinical medical use. Magnetite nanoparticle produced using aqueous co precipitation usually exhibit wide particle size distribution. small super paramagnetic iron oxide nano particles easily permeate the tissues. The physicochemical properties of nano particle such as particle size and zeta potential are important for understanding the interaction of nanoparticles with biological system.[22] Therefore the toxicity of iron nanopraticle depend on their physicochemical properties like particle size, surface properties and its chemical composition.[23]

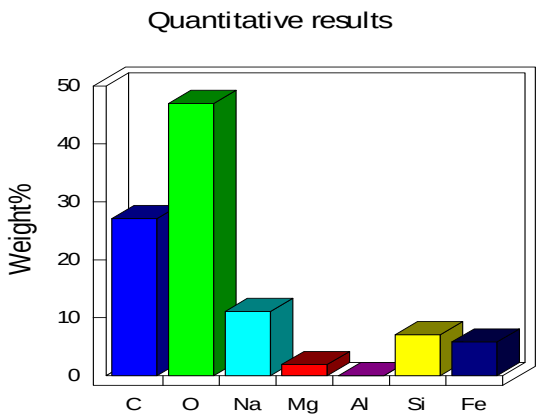

Fig. 3: EDS Quantitative Results of Elements.

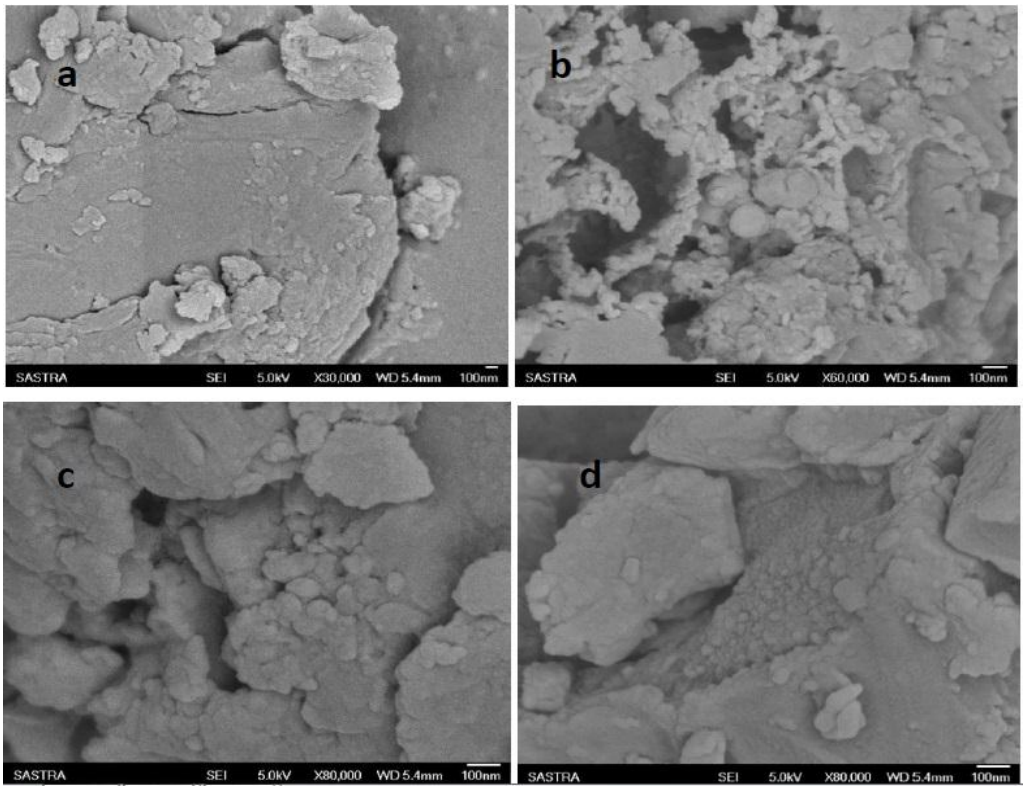

Plate 1

Plate (1a)(1b)(1c)(1d):Field emission scanning electron microscopic picture of prepared ABK drug, image at different magnifications.

Spectroscopic and thermal analysis

The functional groups present on the surface of the ABK drug has been studied using vibration spectroscopy (FT-IR) and showed in Fig. 4. The spectra show various vibration bands at 3430, 2925, 1618, 1385 and 1079 cm^{-1} which correspond to the -OH stretch, -CH2-stretch, -C=O stretch, -O-C-C stretch and so on. These stretching and bending vibration bands confirm the presence of organic moieties on the surface of ABK drug. Similarly, FTIR study was done in Lauha Bhasma Characterization.[24]

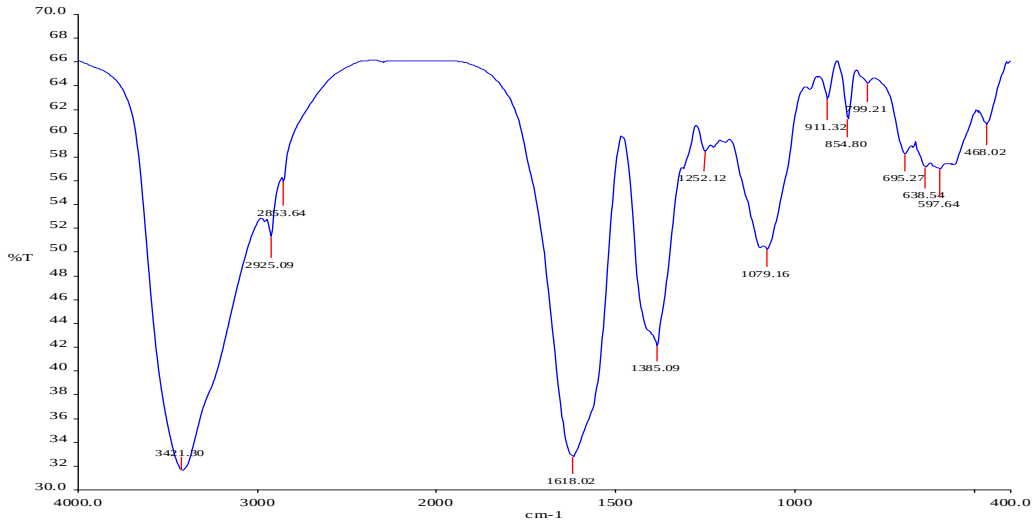

Fig. 4: FT-IR analysis of ABK drug.

TG-DTA analysis

The organic and moisture content of the ABK drug was also elucidated using TG-DTA technique. Fig. 5. Shows the TG-DTA of final ABK drug. It enables us to view the change in the weight of the substance with respect to the temperature over a period of intervals. It is observed that ~20% weight loss is recorded between 100 °C and 200 °C due to moisture presence in ABK drug. The further increase in temperature from 200 °C to 800 °C, there is almost ~35% weight loss recorded. This is due to the organic groups present on the surface of ABK drug which gets decomposed between the temperature 200 °C and 800 °C. The organic groups may be eliminated as carbon-di-oxide, nitrose oxide and so on. The residual percentage of ABK drug beyond 800 °C was found to be only 45% which represents the 100% iron oxide residue. The previous similar study carried out on facile method of synthesizing and characterization of Lauha Bhasma.[25]

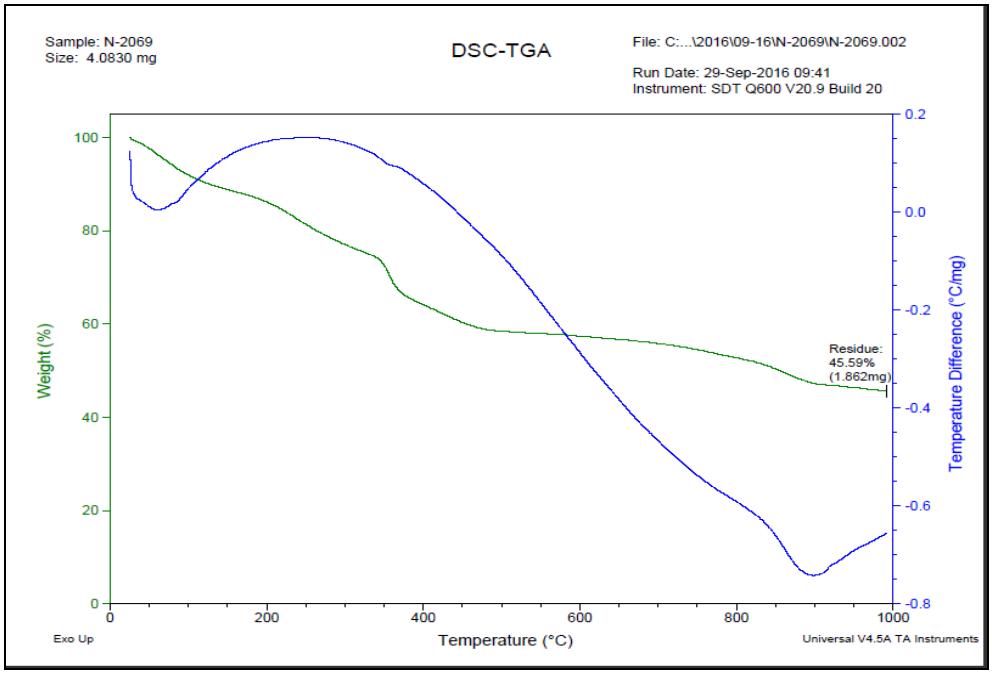

Fig. 5: TG-DTA analysis of ABK.

AAS Analysis

The toxic elements of the ABK drug was detected using AAS technique. Table 2 shows the toxic elements ABK drug. It is also noteworthy to observe that the presence of heavy metal Pb (0.14 PPM), Cd (0.06 PPM), Hg (0.27 PPM) and As (1.85 PPM) were found within the permissible limit of WHO according to XRF and AAS. The activity of heavy metal estimation performed by AAS indifferent preparation of Iron.[26]

Table 2: Heavy metal analysis of ABK.

SN	Elements	Results	WHO Permissible limit
1	Mercury(Hg)	0.2755ppm	1ppm
2	Cadmium (Cd)	0.06ppm	0.3ppm
3	Arsenic(As)	1.8549ppm	3ppm
4	Lead (Pb)	0.014ppm	10ppm

CONCLUSION

The clinical use of metal and mineral drugs was limited due to misconception among the scientific community. Hence, the scientific validation of ABK has been carried out as evidence in this study for widespread among public and scientific community in future. The trial drug ABK has successfully prepared through the well-established siddha *shastrial* method. The prepared ABK drug has been validated through chemical characterization by using appropriate chemical analytical methods.

XPS pattern showed the presence of organic matter on the surface of the drug suggest it act as coating material in the surface of the metallic compound. Therefore, the ABK pharmacologically act as well absorbent and. adsorbent. The microscopically appearance of SEM with EDS showed the nano particle size of ABK form association with organic molecules and enhances the biocompatibility of the drug.

XRD pattern of purified iron and mandooram showed the low intense peak of iron due to removal of iron oxide after purification process and also the ABK showed the formation of Fe_2O_3. XRF study revealed that the increased iron content in purified form confirms the removal of oxides from the raw materials and the ferric form iron in ABK is 77.23%. The organic and moisture content of the ABK drug was also elucidated using TG-DTA technique. AAS study also confirms the absence of toxic heavy metals. Hence, it is concluded that the ABK is biocompatible safe internal drug to treat illnesses as per the reputed siddha text. Further, studies like cell-viability, cell-uptake, bio-distribution and mechanism of action have to be studied in future to know about biological activity of ABK drug.

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CONFLICT OF INTEREST

None.

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