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ANALYTICAL STANDARDIZATION OF BALADI MANDURAM

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ABSTRACT

Rasa Shastra is considered as the pharmaceutical branch of Ayurveda which mainly deals with the metallic and herbo-mineral preparations. *Baladi Manduram* is a unique herbo-mineral preparation mentioned in *Rasa Kamadhenu* and *Rasa Yoga Sagara* - II *Pakaradi* Rasa for the management of Amlapitta, which contains *Mandura Bhasma* as the chief ingredient along with the herbal drugs like *Balamula, Satavarimula, Erandamula, Yava, Guda, Jiraka, Pippali, Twak, Ela, Patra* and *Nagakesara. Shodhana, Bhavana, Marana, Churna nirmana* and *Paka* are the main pharmaceutical procedures involved in the preparation of *Baladi Manduram*. To assure the safety and to understand about the identity, form, particle size and surface morphology of the above formulation, it was subjected to analysis through various techniques like X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDS), Particle size analysis (PSA), Zeta Potential (ZP), UV-Spectroscopy, Fourier transform Infra-Red spectroscopy (FTIR) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). XRD of *Baladi Manduram* showed major peaks of Fe_2O_3 (Iron oxide) and K_2SO_4 (Potassium Sulphate), minor peaks of $CaFeO_4$ (Calcium iron oxide). SEM micrographs showed the cluster of bigger and smaller particles. EDS analysis confirmed the significant presence of elements viz. Fe-49.65%, O- 27.91%, Si- 8.26%, Ca- 5.48%, and Tb- 3.62%. Particle size was found to be 11 nm with Zeta Potential of 22.8 mV. UV- Spectrum of *Baladi Manduram* showed maximum absorption at 283 nm. FTIR test showed 16 peaks between the wavelength 3696.60cm^{-1} to 598.02 cm^{-1}. ICP analysis revealed Iron as main constituent with 89659.20 ppm.

Keywords: *Baladi Manduram*, *Rasa Kamadhenu*, Analytical studies, Safety.

INTRODUCTION

Rasa oushadies are considered superior because of their innate qualities like quick action, lesser dose, tastelessness and prolonged shelf life[1]. Baladi Manduram is one such Rasa Oushadi described in classics like Rasa Kamadhenu[2] and Rasa yoga sagara- II Pakaradi Rasa[3] for the management of Amlapitta. It contains Mandura Bhasma as the main ingredient along with herbal ingredients like Balamula (*Sida cardifolia*), Satavarimula (*Asparagus racemosus*), Erandamula (*Ricinus communis*), Yava (*Hordeum vulgare*), Guda (Jaggery), Jiraka (*Cuminum cyminum*), Pippali (*Piper longum*), Twak (*Cinnamomum zeylanicum*), Ela (*Eletteria cardomum*), Patra (*Cinnamomum tamala*) and Nagakesara (*Mesua ferrua*). Mandura Bhasma is a mineral preparation obtained from the repeated incineration of Mandura (Rust iron). Use of metals and minerals in medicine is often associated with toxicity, but Ayurveda made them into biocompatible form by certain detoxification processes like Shodhana, Marana, Bhavana etc. which removes the toxic potential from metals, minerals and imparts them with therapeutic efficacy of a high grade.

Classical texts have enumerated certain tests which ensure the proper transformation of basic metal and mineral into bio-absorbable Bhasma form. But, in the present scientific era there is a change in the mind set of patients. Safety of the drug to be administered is at par with its efficacy. Analytical study is mandatory to check the raw samples, intermediary products and final product. The presence of free metal or particles of large size in any formulation can lead to damage of vital organs of the body. Hence highly sensitive modern parameters are employed for gaining information about identity, form, particle size and structure of contents of the formulation. By taking this into consideration, an attempt has been made to analyse Baladi Manduram through X-ray diffraction, Scanning electron microscopy, Energy dispersive X-ray spectroscopy, Particle size analysis, Zeta Potential, UV-Spectroscopy, Fourier transform Infra-Red spectroscopy and Inductively Coupled Plasma – Optical Emission Spectrometry.

MATERIALS AND METHODS

Mandura and Triphala were obtained from local market of Chennai, Tamil Nadu. Balamula, Satavarimula, Erandamula, Yava, Pippali, Jiraka, Twak, Ela, Patra, Nagakesara, Guda and Kumari were obtained from TTD's Sri Srinivasa Ayurveda Pharmacy, Tirupati. Gomutra was collected from the Goshala of ISCKON temple, Tirupati. Entire preparation of Baladi Manduram was carried out in TTD's Sri Srinivasa Ayurveda Pharmacy and Department of Rasa Shastra and Bhaishajya Kalpana, S.V.Ayurvedic College, Tirupati. Requirement for XRD: Model- Powder X-Ray Diffractometer D8 advance, Manufacturer- Bruker Germany. SEM and EDS: Model- EVO MA 15, Manufacturer- Carl Zeiss – Germany; PSA and ZP: Model- Horiba scientific Partical Size and Zeta Potential Analyzer, Manufacturer- Horiba instruments, Irvine, CA 92618 USA; UV- Spectroscopy: Model- Nano drop 8000 Spectrophotometer, Manufacturer- Thermo Scientific, India ; ICP-OES: Model- Agilent 725, Manufacturer- Agilent technologies, USA.

Pharmaceutical process

The main pharmaceutical procedures involved in the preparation of Baladi Manduram are Nirvapa, Bhavana, Marana, churna nirmana and Paka. Mandura was taken and subjected to

Shodhana by Nirvapa in Gomutra Triphala Kashaya for 7 times[4]. Then the Shodhita Mandura was triturated with Kumari Swarasa and subjected to Marana by Gaja puta for 7 times, till the bhasma attains all the Bhasma lakshanas mentioned in the classics[5]. 100 g of Guda was taken, made into paka and fine powders of herbal ingredients (100 g each of Balamula, Satavarimula, Erandamula, Yava; 50g of Pippali and Jeeraka; 8 g each of Twak, Ela, Patra and Nagakesara) and 1264 g Mandura Bhasma were added one by one and heated on moderate flame. After self-cooling, the mixture was dried under sunlight in a tray. Homogenous mixture of Baladi Manduram was filled in capsules of 500mg.

Analysis of Mandura Bhasma using ancient parameters (Bhasma Pariksha)

- Rekhapurnatva[6]: After proper trituration, small amount of bhasma was taken between thumb and index finger. It filled into the fine lines of fingers. Rekhapurnatva was obtained after 6th puta.
- Varitaratwa[7]: After proper trituration, small amount of bhasma was sprinkled on the surface of water. Bhasma being light floated on the surface of water. This was obtained after 7th puta.
- Niswadu Pareeksha: When a small amount of the bhasma was kept on tongue, there was not any feeling of taste / untoward sensation. This was obtained after 7th puta.

Analysis of Baladi Manduram using modern parameters

X-Ray Diffraction (XRD)

Baladi Manduram was subjected to XRD at Department of Physics, Yogi Vemana University, Kadapa, Andhra Pradesh.

Procedure: Sample was powdered in agate mortar to very fine powder and it was mounted in sample tray of machine. X-Ray beam bearing a wavelength of 1.5418740 A° from copper source is passed on the sample. Detector was set to identify diffracted beams between 10 -70 degrees of 2 range. Obtained soft files of XRD consisting values of 2θ and intensity are plotted on a graph (2θ on X-Axis and Intensity of Y-Axis) using "Origin Pro 8.5 SR2" Data Analysis Software. Various compounds consisting similar diffraction pattern were identified by matching their peaks with corresponding JCPDS Crystallographic cards. For even better accuracy and precision, XRD soft files were also analyzed for corresponding phase/entry matching with Crystallographic Open Data base (COD - 20120320) – USA, after plotting values in PANalytical X'pert high score plus software 3.0.0.123, UK.

Scanning Electron Microscopy and Energy dispersive X-Ray spectroscopy

The practical was performed at Department of Physics S.V University, Tirupati.

Procedure of SEM: Specimen of the sample to be analyzed was directly kept on the specimen holder for visualization. As the sample employed has nonconductive nature, the sample surface was coated by carbon by arc melting technique. Then the dried powder was observed under the microscope at 1,000X to 7,000KX and the micrographs were taken with the inbuilt camera.

Procedure of EDS: Electron beam excitation is used in scanning electron microscopes (SEM). A detector is used to convert X-ray energy into voltage signals; this information is sent to a

pulse processor, which measures the signals and passes them on to an analyzer for data display and analysis. The detector used in EDS is often the Lithium drifted Silicon detector which is operated at liquid nitrogen temperatures. Sample of Baladi Manduram was placed on the specimen holder and subjected to Energy-Dispersive X-ray spectroscopy (EDS). When the sample was bombarded by the SEM's electron beam, electrons are ejected from the atoms comprising the sample's surface. The resulting electron vacancies are filled by electrons from a higher state, and an X-ray is emitted to balance the energy difference between the two electrons' states. The X-ray spectrum thus acquired gives the information on the elemental composition of the material under examination.

Particle Size Analysis and Zeta Potential

The practical was conducted at Department of science and Technology, PURSE, S.V. University, Tirupati.

Procedure of PSA: The sample was mixed in water and agitated for 10 minutes. Then it was poured into the sample chamber, where it passes through the laser beam as homogeneous stream of particles. The scattering of light occurs over a wide range of angles upon interacting with the particles in the suspension which are moving by Brownian motion. Based on this scattering pattern of sample, particle size distributions are calculated comparing with appropriate optical model.

Procedure of ZP: A 1% concentration of Baladi Manduram was prepared in distilled water. The particles were well dispersed before analysis and the sample was taken in a 1ml syringe and injected slowly into the capillary cell (cuvette) through the sample port. Care was taken to see that air bubbles are not formed during this process. As the sample comes out from the second port of the capillary cell, the injection process is stopped. This indicates complete filling of the sample into the capillary cell. The sample ports are then covered with lids and the capillary cell was then placed into the sample holder of the zeta sizer instrument for analysis.

UV- Spectroscopy

Practical was performed at Department of science and Technology, PURSE, S.V.University, Tirupati.

Procedure: 5gm of Baladi Manduram was macerated with 100 ml of solvent in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was filtered, taking for UV spectroscopic study. The Spectra was taken at 200-800 nm from the peak obtained the λmax value was calculated.

Fourier Transform Infrared Spectroscopy (FT-IR)

This practical was conducted at Padmavathi Mahila University, Tirupati.

Procedure: Sample was placed in the Potassium bromide plate of FTIR spectrometer and the interference pattern was detected by the infrared detector as variations in the infrared energy level, and the obtained spectral information was calculated.

Inductively Coupled Plasma – optical Emission Spectrometry

This practical was performed at Centre for material for electronics technology (C-MET), department of Electronics and Information technology, Hyderabad.

Procedure: 0.2 g of Baladi Manduram was taken in Teflon tubes and added 6.0 ml of Nitric acid and 2.0 ml of Hydrogen peroxide and allowed for 10 minutes in outside for reaction. Then samples were dissolved using Microwave Digestion System (Anton Paar Multiwave

3000). Then Baladi Manduram solutions were made to 25.0 ml and filtered. These solutions were used for Elemental analysis using ICP-OES instrument.

RESULTS

X-Ray Diffraction Studies (XRD):

Table 1: Showing the details of matching peaks of XRD data for Baladi Manduram:

S.No	Element/Molecule	JCPDS Ref.No	2θ	Intensity	FWHM	H	K	L
1.	Fe_2O_3(Iron oxide)	01-073-2234	24.16 35.65 64.04	31.52 74.00 23.00	0.168 0216 0.12	1 -1 -2	1 1 1	0 0 1
2.	Fe_2O_3(Iron oxide)	00-033-0664	33.15 54.09 49.48 62.45 40.85	5.54 45.00 40.00 30.00 20.00	0.192 0.12 0.144 0.12 0.192	1 1 0 2 1	0 1 2 1 1	4 6 4 4 3
3.	K_2SO_4(Potassium sulfate)	01-071-1912	57.68	7.29	0.384	2	1	3
4.	$CaFeO_4$(Calcium iron oxide)	00-003-0804	72.03	5.56	0.48	4	1	1

Table 2: Showing Crystal details of JCPDS entries:

Name	Iron Oxide (JCPDS- 01-073-2234)
Space group	R-3c
Crystal System	Rhombohedral
Cell Parameters	a = 5.4240A˚ b= 5.4240 A˚ c = 5.4240 A˚
Z	2.0

Name	Iron Oxide (JCPDS- 00-003-0664)
Space group	R-3c
Crystal System	Rhombohedral
Cell Parameters	a = 5.0356A˚ b= 5.0356 A˚ c = 13.7489A˚
Z	6.0

Name	Potassium sulfate
Space group	P63/mmc
Crystal System	Hexagonal
Cell Parameters	a = 5.9470A˚ b= 5.9470A˚ c = 8.3750 A˚
Z	2.0

Name	Calcium Iron Oxide
Space group	Pnma
Crystal System	Orthorhombic
Cell Parameters	a = 5.4200A˚ b= 14.7520 A˚ c = 5.5940 A˚
Z	-

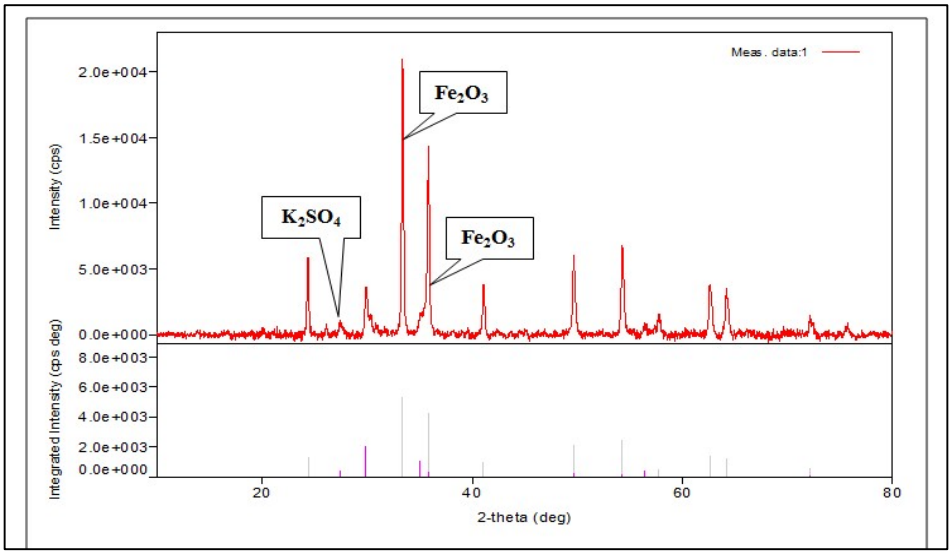


Figure 1: Showing XRD peaks of Baladi Manduram

XRD of Baladi Manduram shows major peaks of **Fe_2O_3** (Iron oxide) compound with Rhombohedral structure and **K_2SO_4** (Potassium Sulphate) compound with Hexagonal structure. Minor peaks showed presence of **$CaFeO_4$** (Calcium iron oxide) compound with Orthorhombic structure. Fe_2O_3 peak was detected at diffraction angle of 24.16, 35.65, 64.04, 33.15, 54.09, 49.48, 62.45, and 40.85. K_2SO_4 peak was detected at diffraction angle of 57.68, and $CaFeO_4$ peaks at 72.03.

Scanning Electron Microscopy:

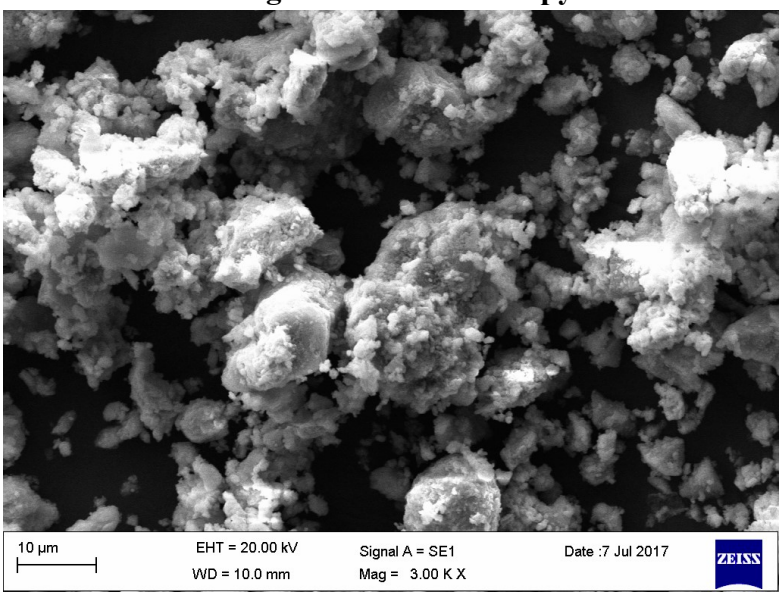


Figure 2: Showing SEM result of Baladi Manduram (Mag. 3KX)

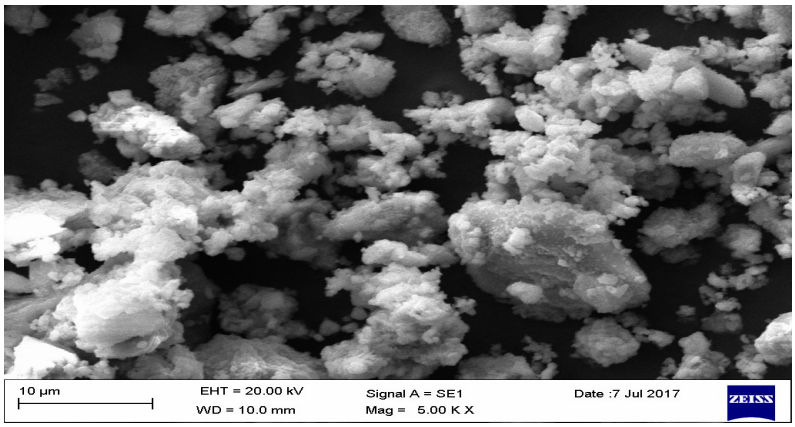


Figure 3: Showing SEM result of *Baladi Manduram* (Mag. 5KX)

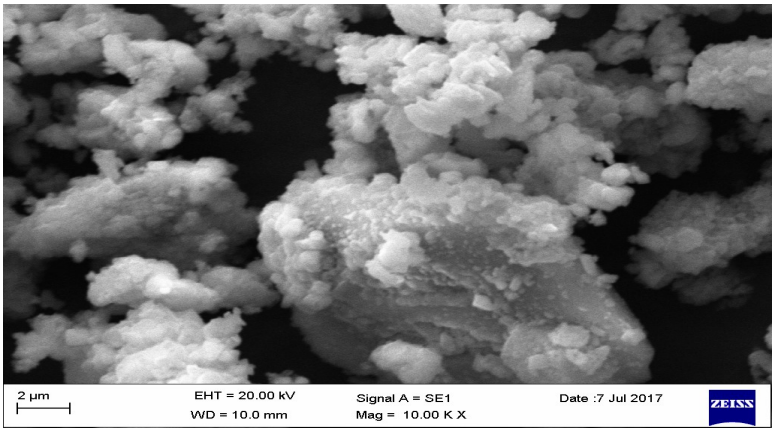


Figure 4: Showing SEM result of Baladi Manduram (Mag. 10KX)

SEM micrographs of Baladi Manduram showed the cluster of bigger and smaller particles. Smaller particles of spherical shape were seen in sticking to the surface of bigger particles.

Energy Dispersive X-Ray Spectroscopy:

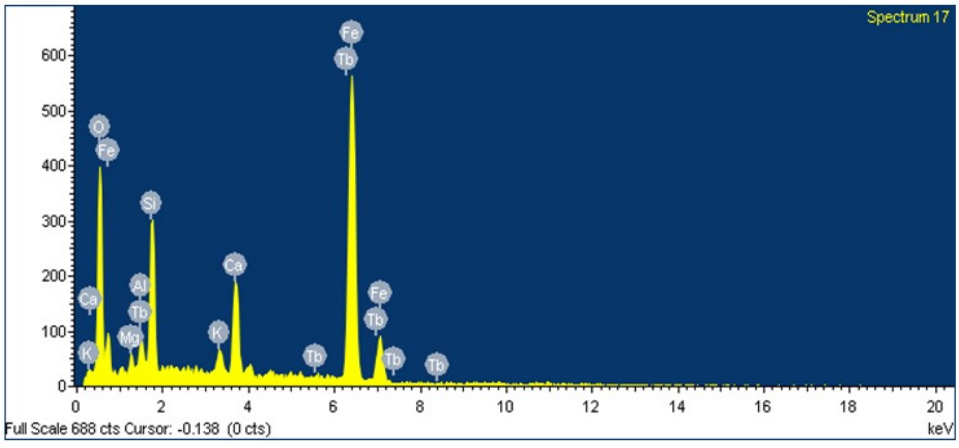


Figure 5: Showing EDS graph of Baladi Manduram

EDS analysis of Baladi Manduram confirmed the presence of following elements viz. **O- 27.91%, Mg- 2.62%,** Al- 1.48% **Si- 8.26%,** K- 1.26%, **Ca- 5.48%, Fe-49.65%,** Tb- 3.62%.

Table 3: Showing the quantity of all the elements in Baladi Manduram

Element	Weight%
O K	27.60
Mg K	2.62
Al K	1.48
Si K	8.26
K K	1.26
Ca K	5.48
Fe K	49.65
Tb L	3.65
Totals	100.00

Particle Size Analysis:

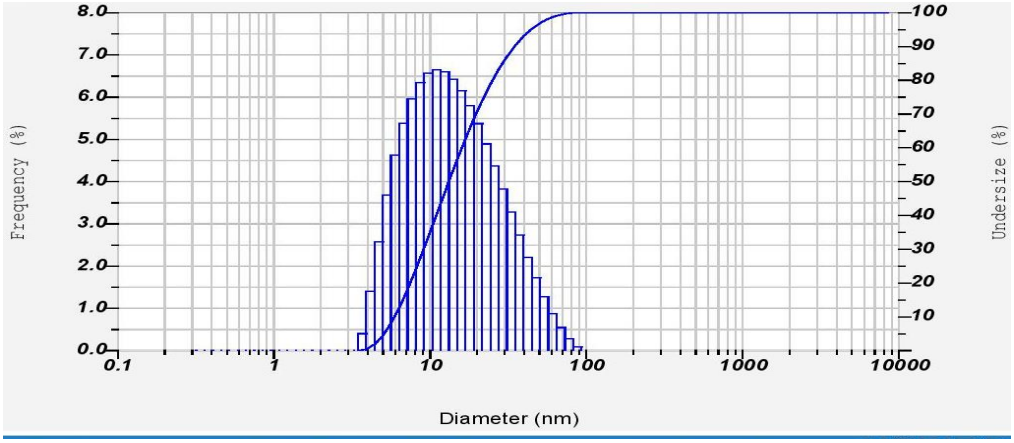
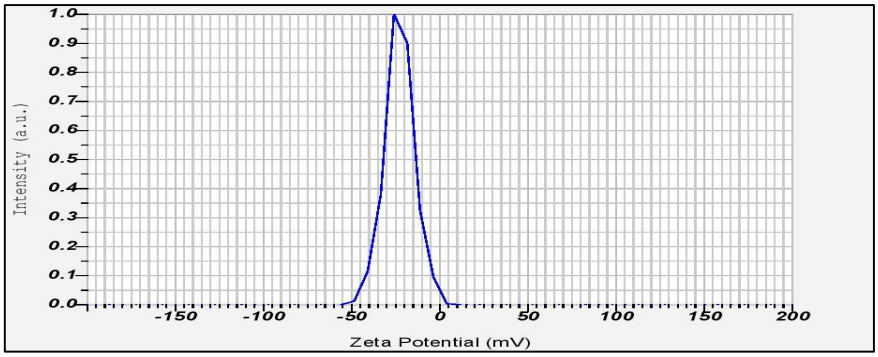


Figure 6: Showing the result of Particle size analysis of Baladi Manduram

The mean particle size of Baladi Manduram is **11 nm.**

Zeta Potential



Graph 7: Showing Zeta potential distribution of Baladi Manduram

Baladi Manduram sample showed a Zeta potential value of -22.8 **mV,** which indicates moderate colloidal stability.

UV- Spectroscopy:

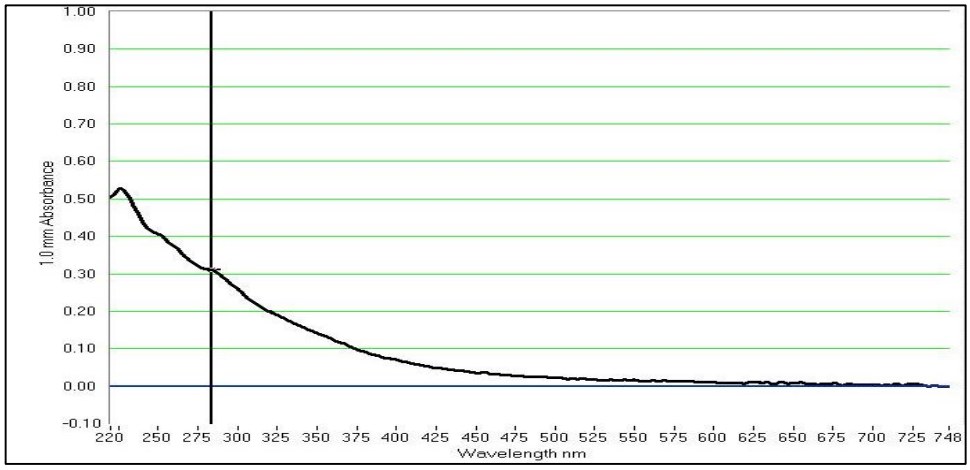


Figure 8: Showing UV-Spectrum of Baladi Manduram

UV - Spectrum of Baladi Manduram showed maximum absorption at **283 nm.**

Fourier Transform Infrared Spectroscopy (FT-IR):

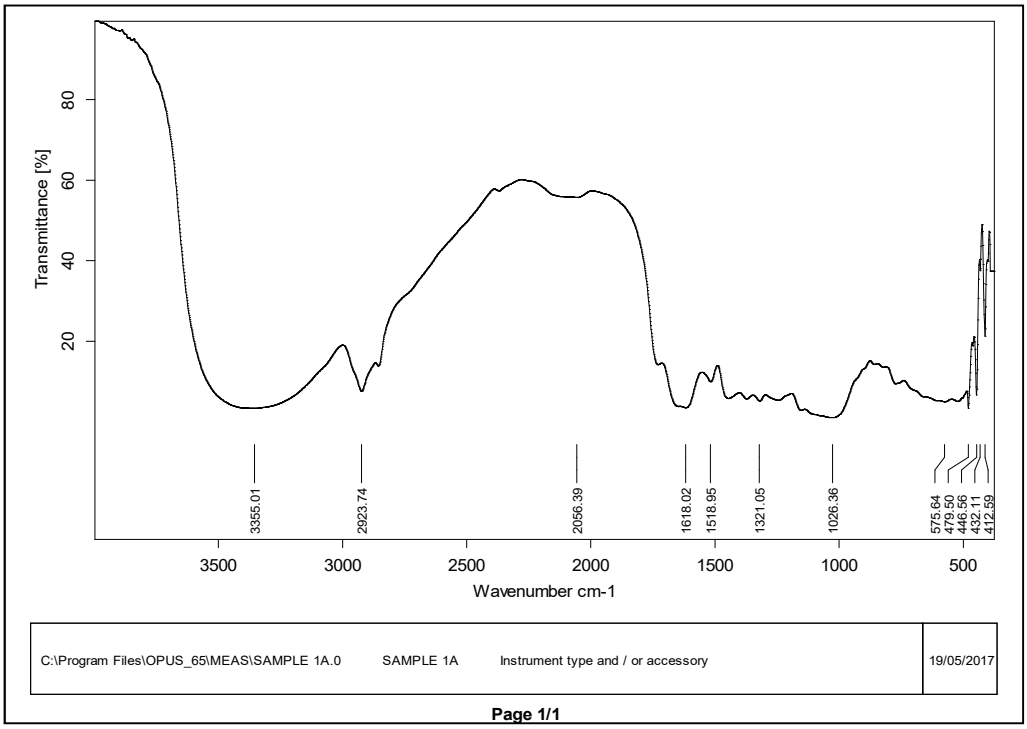


Figure 9: Showing various peaks obtained in FTIR analysis of Baladi Manduram

Table 4: Showing details of Peaks obtained in FTIR analysis of Baladi Manduram.

Sample Name	No. of Peaks	Wave length
Baladi Manduram	16	3917.69, 3877.22, 3825.87, 3706.72, 3696.60, 3634.12, 3374.17, 2938.74, 2380.73, 2344.38, 1753.47, 1600.24, 1528.15, 1434.88, 1030.61, 598.02

Table 5: Showing various peaks obtained in FTIR analysis of Baladi Manduram and their correlation with compounds

S.No.	Peak	Actual peak	Bond	Type of bond	Specific type of bond	Appearance
1.	3600-3700cm^{-1}	3696.60cm^{-1} 3634.12cm^{-1}	O-H	Alcohol Phenols	low concentration	-
2.	3200-3400cm^{-1}	3374.17cm^{-1}	O-H	Alcohol Phenols	High concentra-tion	Broad
3.	2400-3200cm^{-1}	2938.74cm^{-1}	N-H	Ammonium ions	any	Multiple broad peaks
4.	1700cm^{-1}	1753.47cm^{-1}	C-N	C=N	any	Similar conjugation effects to C=O
6.	1600cm^{-1}	1600.24cm_{-1}	C-C	Aromatic C=C	Any	Weak to strong(usually 3 or 4)
7.	1020-1220cm^{-1}	1030.61cm^{1}	C-N	Aliphatic amines	Any	Often overlapped
8.	540-760cm^{-1}	598.02cm^{-1}	C-X	Chloro Alkanes	Any	Weak to medium

Inductively Coupled Plasma – optical Emission Spectrometry:

Table 6: Showing the result of ICP-OES analysis of Baladi Manduram.

S.No.	Name of the elements analyzed	Tests results in ppm
1.	Silver	16.01
2.	Arsenic	4.10
3.	Boron	13.24
4.	Calcium	1420.12
5.	Cadmium	5.31
6.	Chromium	97.62
7.	Copper	238.90
8.	Iron	89659.20
9.	Mercury	6.08
10.	Potassium	1232.91
11.	Magnesium	953.73
12.	Manganese	175.06
13.	Sodium	122.07
14.	Phosphorus	468.49

15.	Lead	246.69
16.	Sulphur	614.75
17.	Selenium	ND
18.	Tin	2.65
19.	Zinc	52.39

DISCUSSION

Analytical study is an essential part of any research work. It provides us with experimental data (qualitative and quantitative) and makes us know about certainty of our assumptions and prevents from miss interpretations. It gives us the knowledge about identity, size, structure of chemical constituents and physical properties. It hints us about toxic properties of drugs, if any.

X-ray diffraction has been in use in two main areas, for the finger print characterization of crystalline materials and the determination of their structure. Each crystalline solid has its unique characteristic X-ray powder pattern, which may be used as a "fingerprint" for its identification. Once the material has been identified, X- ray crystallography may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the inter-atomic distance and angle etc. X-ray diffraction is one of the most important characterization tools used in solid state chemistry and materials science. Size and the shape of the unit cell for any compound can be detected most easily using the diffraction of X-rays. Major peaks of Fe_2O_3 and K_2SO_4 and minor peaks of $CaFeO_4$ were seen in XRD reprint. Mandura is considered as rusted iron, its chemical formula is $Fe_2O_3.H_2O$. During shodhana and Marana process the water portion of Mandura may get evaporated and only Fe_2O_3 (ferric oxide) remains. Presence of Fe_2O_3 indicates Mandura Bhasma in Baladi Manduram. The other minor peaks observed were due to formation of Herbo mineral complex.

Scanning electron microscopy (SEM) is an analytical technique to know the surface morphology of the drug. It uses electron beam rather than light to form a Figure. It is capable of producing high resolution figures of a sample surface, which means that closely spaced features can be examined at a high magnification. Due to the manner in which the Figure is created, SEM Figures have a characteristic three dimensional appearance and are useful for determining the surface structure of the sample i.e. topography. It can magnify objects to extreme levels where even structure of nano particles could be clearly visible. The distribution of particles in Baladi Manduram as clusters of bigger and smaller particles may be due to accumulation of herbal drugs over the surface of Mandura Bhasma. Surface of Baladi Manduram appeared smooth. It may due to the involvement of procedures like Nirvapa, Bhavana and Marana.

Energy-Dispersive X-ray spectroscopy (EDX) is an analytical technique used for elemental analysis or chemical characterization of a sample. It relies on the investigation of an interaction of some source of X-ray excitation and a sample. EDS of Baladi Manduram confirmed the presence of elements like Iron and oxygen which are present in Mandura. The presence of other elements like Calcium, Magnesium and Silica may be due to the addition of herbal ingredients.

The size of the particles in the drug plays major role in its therapeutic action and efficacy. Particle size and surface area of solid drug are inversely related to each other. The mean particle size of the particles of Baladi Manduram is 11nm. The nano size of drug is indicative of its quick absorption and faster dispersion into body resulting in better therapeutic efficacy. Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles, and is one of the fundamental parameters known to affect stability. The Zeta Potential (mean) value of Baladi Manduram was found to be-22.8mV which indicates moderate colloidal stability.

UV-Spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups with the molecule. Electromagnetic spectrum of U.V region is from 190 to 400 nm whereas for visible region it is 400-800 nm. UV- Spectrum of Baladi Manduram showed maximum absorption at 283 nm.

FTIR was performed to detect the presence of functional groups or organic legends in Baladi Manduram. Infrared spectroscopy deals with the infrared region of the electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. When infrared light or radiation hits a molecule, the bonds in the molecule absorb the energy of the infrared and respond by vibrating. Baladi Manduram showed 16 peaks between the wave length 3696.60cm^{-1} to 598.02 cm^{-1}. Absorption peaks of alcohols and phenols with high and low concentration were observed between 3600-3700 cm^{-1} and 3200-3400 cm^{-1}. Wave lengths 1753.47cm^{-1} and 1030.61 cm^{-1} represent C-N bonds with Aliphatic amines. A single peak with aromatic C-C bond and C-X bond with chloroalkanes were obtained near 1600.24 cm^{-1} and 598.02 cm^{-1} wave lengths.

ICP-OES is one of the most powerful and popular analytical tool for the determination of trace elements in a sample. It is very useful for standardization as well as to develop analytical profile. ICP–OES analysis of Baladi Manduram showed Iron as main constituent in 89659.20 ppm. This confirms the presence of Iron in Mandura bhasma, Bala, Pippali, Satavari and Nagakesara. 1420.12 ppm of Calcium, 1232.91 ppm of Potassium 953 ppm of Magnesium, 614.75 ppm of Sulphur and 468.49 ppm of Phosphorous was present. This may be due to the presence of these elements in herbal drugs like Kumari, Bala, Satavari and Jiraka. Toxic elements like Selenium were not detected.

CONCLUSION

The present study confirms the fact that Baladi Manduram is a herbo-mineral compound which mainly consists of Fe_2O_3 as the major phase. The particle size indicates the presence of nano sized particles in this formulation. Presence of other micro essential elements may be due to the herbal ingredients used in the process of preparation and from the component drugs of Baladi Manduram. Hence these entire analytical tests justify the structural and chemical composition of the compound indicating its safety and efficacy for a safe therapeutic approach.

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