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ANALYTICAL STANDARDIZATION OF *GANDHAKAKALPA*

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ABSTRACT

Rasa shastra is a branch of medicine which deals with preparation of drugs of metals and minerals having wide range of therapeutic efficacy and possessing innate qualities like quick action, less dose, tastelessness, prolonged shelf life and better palatability. *Gandhakakalpa* is one of the unique formulations in which *Shuddha Gandhaka* and *Madhuka taila* are the main ingredients. *Shodhana*, *Murchchana* and *Mardana* are the main pharmaceutical procedures involved in the preparation of *Gandhakakalpa*. 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 analytical parameters 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 InfraRed spectroscopy (FTIR) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). XRD of *Gandhakakalpa* shows major peaks of S_8. SEM micrographs showed spherical and glomerulated particles; particle size was found to be 14.7nm and its Zeta potential is -32.7 mV, UV spectrum of *Gandhakakalpa* showed maximum absorption at 261nm and 268nm, FT-IR analysis showed 11 peaks between the wavelengths 3006.72 – 407.54 cm^{-1} and ICP – OES analysis revealed Sulphur as main constituent in 96164 ppm.

KEYWORDS: *Gandhakakalpa*, Modern Analytical parameters, Safety, Toxicity.

INTRODUCTION

Analytical study plays an important role in the standardization of the drugs. *Ayurveda*, the ancient system of medicine is gaining recognition throughout the world and many herbal, metal and mineral drugs are now clinically tested and accepted. However, one of the impediments in the acceptance of the ancient systems of medical preparation is the lack of standard quality control profiles. The quality of the drugs, that is, the profile of the constituents in the final product has implication in efficacy and safety.

Gandhakakalpa is one of the unique formulations mentioned in *Basavarajeeyam* 18th chapter 20th sloka.[1] It contains the ingredients *Shuddha Gandhaka* and *Madhuka taila*. All these dravyas possess therapeutic properties indicated in the management of several diseases.

Shodhana of *Gandhaka* is done by *puta* method mentioned in *Rasendrasarasangraha.*[2] *Murchchana* of *Madhuka taila* is done by the method mentioned in *Bhaishajya ratnavali*.[3]

Therefore modern analytical techniques are expected to help in circumventing this problem. Hence highly sensitive modern parameters like 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 were employed for gaining information about identity, form, particle size, surface morphology, structure and contents of the formulation.

MATERIALS AND METHODS

Gandhaka and *Madhuka taila* were collected from Vijayawada. Entire preparation of *Gandhakakalpa* was carried out in Department of Rasa Shastra and Bhaishajya Kalpana, TTD's S. V. Ayurvedic College and Sri Srinivasa Ayurveda Pharmacy, TTD, Tirupati. Requirement for XRD: Model- Powder XRay Diffractometer D8 advance, Manufacturer Bruker Germany. SEM: 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 *Gandhaka kalpa* are *Shodhana*, *Murchchana* and *Mardana*. *Ashuddha Gandhaka* was taken and subjected to *shodhana* by *puta* method. *Murchchana* is done for *Madhuka taila*. *Shuddha Gandhaka* is triturated with *Murchchitha Madhuka taila* and *vati* of uniform size of 500mg was prepared in *Khalwayantra.*

Analysis of *Gandhakakalpa* using modern parameters

PH[4]

PH is defined as the common logarithm of the reciproacal of the hydrogen ion concentration expressed in grams per litre.

PH value of *Gandhakakalpa* is 6 which is slightly acidic in nature.

X-Ray Diffraction (XRD*)*

Gandhakakalpa was subjected to XRD at Department of Physics, Yogi Vemana University, Kadapa and 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 analysed for corresponding phase/entry matching with Crystallographic Open Data base (COD - 20120320) – USA, after plotting values in Analytical X'pert high score plus software 3.0.0.123, UK.

Scanning Electron Microscopy

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

Procedure of SEM

Specimen of the sample to be analysed 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 300X, 600X, 1.20X and 2.40KX and the micrographs were taken with the inbuilt camera.

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 sonicated 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 *Gandhakakalpa* 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: 5g of *Gandhakakalpa* 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 261 and 268 nm from the peak obtained the λ max 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 *Gandhakakalpa* 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 Par Multi wave 3000). Then *Gandhakakalpa* 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 *Gandhaka kalpa.*

S. No.	Element/Molecule	JCPDS Ref. No.	2θ	Intensity	FWHM	h k l
1.	S_8	00-008-0248	23.083	100	0.2880	2 2 2
			27.769	60	02160	0 4 0
			25.879	40	0.2880	0 2 6
			28.681	25.0	0.2160	3 1 3
			26.750	25.0	0.2880	3 1 1
			21.874	12.0	0.1920	2 2 0

Table 2: Showing Crystal details of JCPDS entries.

Space group	**Fddd**
Crystal system	**Orthorhombic**
Cell parameters	**a = 10.4500A^0, b = 12.8400A^0, c= 24.4600A^0**
Z	**128**

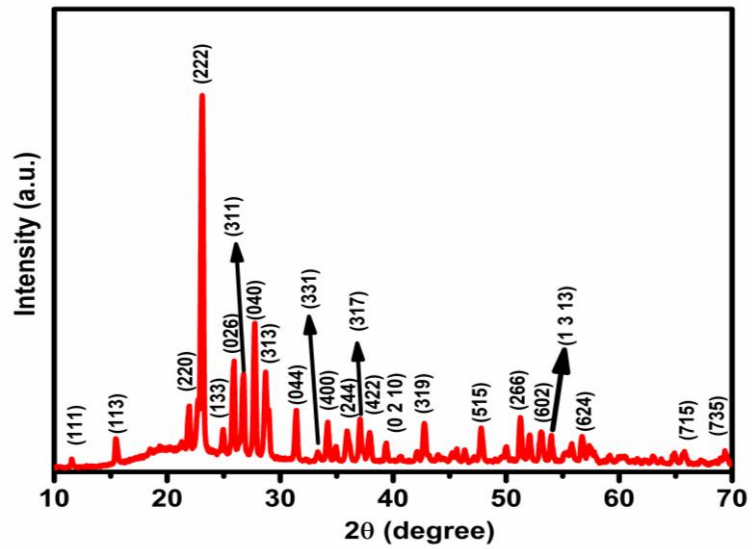

Figure 1: Showing XRD peaks of *Gandhakakalpa.*

XRD of Gandhakakalpa shows that major peaks are of S8 (Sulphur) with Orthorhombic structure at diffraction angles at 23.083, 27.769, 25.879, 28.681, 26.750, 21.874.

Scanning Electron Microscopy

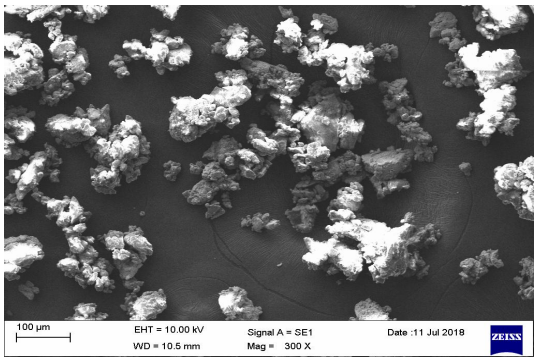

Figure 2: Showing SEM result of Gandhakakalpa (Mag. 300X).

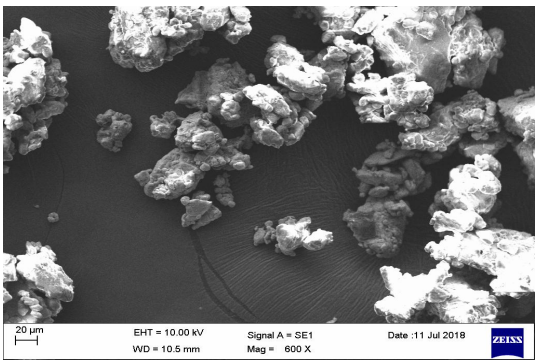

Figure 3: Showing SEM result of Gandhakakalpa (Mag 600X).

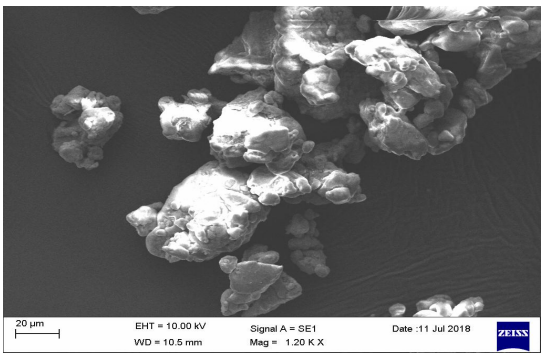

Figure 4: Showing SEM result of Gandhakakalpa (1.20KX).

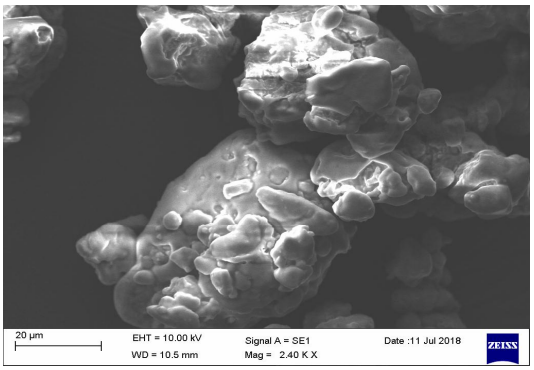

Figure 5: Showing SEM result of *Gandhakakalpa* (2.40KX).

Particle Size Analysis

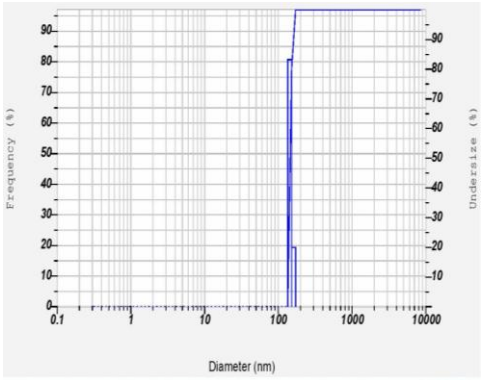

Figure 6: Showing the result of Particle size analysis of *Gandhakakalpa.*

The mean particle size of *Gandhakakalpa* is 14.7nm.

Zeta Potential

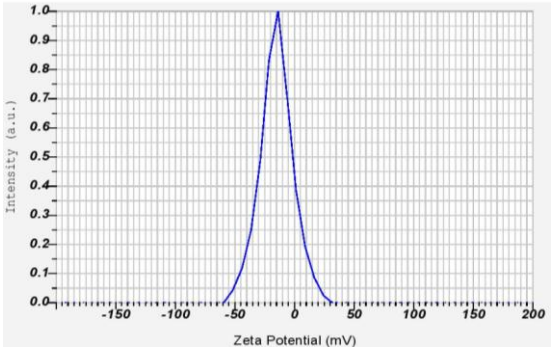

Figure 7: Showing Zeta potential distribution of *Gandhakakalpa*.

Gandhakakalpa sample showed a zeta potential of -32.7mv, which indicates high colloidal stability.

UV-Spectroscopy

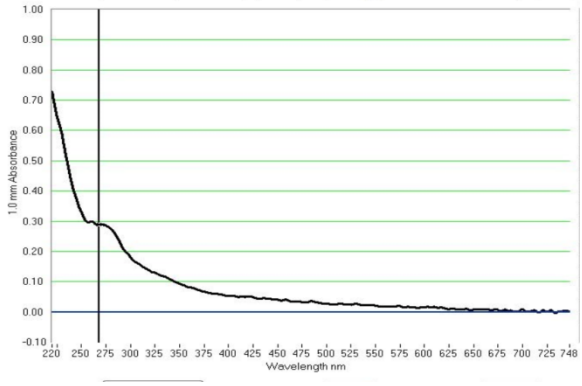

Figure 8: Showing UV-Spectrum of *Gandhakakalpa.*

UV-Spectrum of *Gandhakakalpa* showed maximum absorption at 261 nm and 268nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

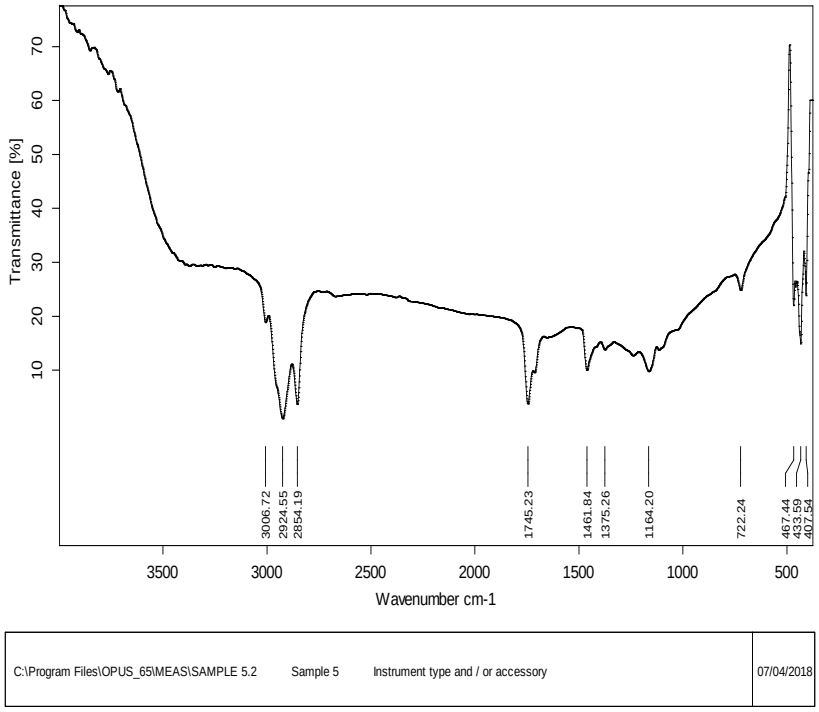

Figure 9: Showing various peaks obtained in FTIR analysis of *Gandhakakalpa.*

Table 3: Details of Peaks obtained in FTIR analysis of *Gandhaka kalpa.*

Sample Name	No. of Peaks	Wave number
Gandhaka kalpa	11	3006.72, 2924.55, 2854.19, 1745.23, 1461.84, 1375.6, 1164.20, 722.24, 467.44, 433.59, 407.54

Table 4: Various peaks obtained in FTIR analysis of *Gandhaka kalpa* and their correlation with compounds.

	Actual peak	Bond	Type of bond	Appearance
1.	3006.72	C-H	Alkenes	Moderate
2.	2924.55	C-H	Alkane	Medium to Strong
3.	2854.19	C-H	Alkane	Medium to Strong
4.	1745.23	C=O	Esters	Strong
5.	1461.84	-CH3	Alkanes	Moderate
6.	1375.26	-CH3	Alkanes	Moderate
7.	1164.20	C-O	Alcohols, Ethers, esters Carboxylic acid, Anhydrides	Strong
8.	722.24	C-H	Alkenes	Strong
9.	467.44	--	---	Not found
10.	433.59	--	---	Not found
11	407.54	--	---	Not found

Inductively Coupled Plasma – optical Emission Spectrometry

Table 5: Showing the result of ICP-OES analysis of *Gandhaka kalpa.*

S. No.	Name of the elements analysed	Tests results in ppm
1.	Silver	Not detected
2.	Arsenic	Not detected
3.	Barium	7.04
4.	Calcium	323.13
5.	Cadmium	Not detected
6.	Chromium	Not detected
7.	Copper	1.97
8.	Iron	208.05
9.	Aluminium	233.69
10.	Potassium	578.12
11.	Magnesium	104.29
12.	Manganese	6.69
13.	Sodium	50.56
14.	Nickel	Not detected
15.	Strontium	0.96
16.	Selenium	Not detected
17.	Vanadium	Not detected
18.	Zinc	10.07
19.	Sulphur	96164

DISCUSSION

Analytical study is a process which helps in identification of quantitative and qualitative data of a substance, the components of a solution or mixture, or the determination of the structures of chemical compounds. It is an essential part of any reaearch work. 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. 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 S_8 were seen in XRD.

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 *Gandhaka kalpa* are spherical and agglomerulated particles at 300X, 600X, 1.20KX, 2.40KX magnifications.

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 *Gandhaka kalpa* is 14.7nm. 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 sattraction between particles, and is one of the fundamental parameters known to affect stability. The Zeta Potential (mean) value of *Gandhaka kalpa* was found to be -32.7 mV which indicates high 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 *Gandhaka kalpa* showed maximum absorption at 261 nm and 268nm.

FTIR was performed to detect the presence of functional groups or organic legends in *Gandhakakalpa.* 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. *Gandhakakalpa* showed 11 peaks between the wave length 3006.72cm-1 to 407.54 cm-1. FT- IR analysis of *Gandhakakalpa* reveals the presence of functional groups CH, C=O, C-C, -CH3, C-O, C-H, i.e. Alkanes Alkenes. Esters, Alcohols, Ethers, Esters, Carboxylic acids, Anhydrides.

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. The ICP-OES analysis of *Gandhakakalpa* reveals the following elements (in PPM units) i.e. Sulphur 283610, Barium 7.04, Calcium 323.13, Copper 1.97, Iron 208.05, Aluminium 233.69, Potassium 578.12, Magnesium 104.29, Manganese 6.69, Sodium 50.56, Strontium 0.96, Zinc 10.07, The heavy metals like Lead, Cadmium, Selenium, Vanadium and Arsenic were not detected in the sample which were confirmed by XRD analysis report.

CONCLUSION

The present study confirms the fact that *Gandhakakalpa* is a herbo-mineral compound which shows the presence of nano sized particles. Presence of other micro essential elements may be due to the herbal ingredients used in the process of preparation. These entire analytical tests justify the structural and chemical composition of the compound indicating its safety and efficacy.

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