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ANALYTICAL STUDY OF GANDHAKAAJEERNA BADDHO RASA THROUGH XRD, SEM, EDS, ZP AND PA

[1]Kumar Saikrishna, [2]Ch. Sridurga.

[1]PG scholar,Dept of Rasa Shastra and Bhaishajya Kalpana,S.V.Ayurveda College,T.T.D, Tirupati.

[2]Associate professor, Dept. of Rasa Shastra and Bhaishajya Kalpana, S.V.Ayurveda College, T.T.D,Tirupati

ABSTRACT :

Rasa Shastra deals with medicinal aspects of metals, minerals, precious stones and poisonous plant drugs. It has been serving humanity from centuries with its unique metallic and herbo-mineral formulations. The *Rasaushadhies* have wide range of therapeutic efficacy and innate qualities like quick action, less dose, palatability, and prolonged shelf life. "*Gandhakaajeernabaddho Rasa"* (GABR) is one such herbo-mineral formulation described to have ability to cure almost all types of diseases when given with suitable *anupana* (vehicle). The main ingredients are *Parada, Gandhaka, Kakamachi, Tambula, Dathura* and *Meghanada.* In this study the drug GABR was prepared and analysed through X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy dispersive X-ray analysis (EDS/EDX), Zeta potential (ZP) and Particle size analysis (PA). XRD revealed that the GABR consists of Hgs (Meta Cinnabar) majorly and Sulphur in minor without any free mercury traces. SEM revealed that the GABR contains Mercury 6.25%, Sulphur 44.95%, Carbon 41.75%, Oxygen 6.64% and Potassium 0.44% by weight. ZP mean was -86.3mV which indicates the high colloidal stability. The mean particle size of the particles is 7nm with standard deviation of 0.47 nm. The details analytical study (i.e, XRD, SEM, EDS, ZP and PA) of *Gandhakaajeernabaddho Rasa* will be discussed in the full paper.

Key words: *Gandhakaajeernabaddho Rasa,* X-ray diffraction, Scanning electron microscope, Energy dispersive X-ray analysis, Zeta potential, Particle size analysis.

INTRODUCTION: Ayurveda drugs are time tested for their efficacy but, Now-a-days there is an apprehension among the general public regarding the safety of these metallic & mineral preparations. As per modern science heavy metals causes damage to vital organs like Liver, Kidney when used internally. Ancient Acharyas were aware of this and had described the detailed deleterious effects likely to be produced by intake of impure/ not purified (Ashudha) and improperly processed metalic/mineral drugs. To remove these deleterious effects and to impart therapeutic properties in these minerals & metals, specialised pharmaceutical process were designed and described under *Shodhana, Marana, Jarana, Murchana etc*. To establish these potent drugs as safe, non-toxic and efficacious, scientific data should be produced through latest tests that can trace the presence of free metals which can damage vital organs of the body. Sophisticated highly sensitive modern tools are available to know the elemental composition, structure of the contents, identity and particle size. Considering these an effort has been made to analyze a unique Rasaoushadhi- *GandhakaajeernaBaddho Rasa* through X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy dispersive X-ray analysis (EDS/EDX), Zeta potential (ZP) and Particle size analysis (PA).

Pharmaceutical process: The main ingredients of GABR are *Parada, Gandhaka, Kakamachi, Tambula, Dathura* and *Meghanada*[1]. The pharmaceutical process involves procedures like *Shodhana of Parada, Gandhaka, Dravikarana, swarasnirmana and Jarana*. *Shodhana* is done for *Parada* (Mercury) and *Gandhaka* (Sulphur)[2,3]. *Swarasas* of freshly collected *Kakamachi, Nagavalli, Dhatura* and *Meghanada* were prepared as per the requirement [4].*Shuddha Parada* and *Shuddha Gandhaka* were taken in to specially designed *Yantra* and were heated on *Mandagni* till *Gandhakanirdhoomavastha*. The homogenous mixture that was formed after *Gandhakanirdhoomavastha* stage was boiled with *Swarasa* of *Kakamachi, Nagavalli, Dhatura* and *Meghanada* one after the other continuously till the completion of *Gandhakajarana* .

- In the chief reference it was said that repeated adding of *Swarasa*s and heating till their total evaporation should be done up to the completion of *Jarana*. Here to appreciate completion of *Jarana*, Vaidya Lolla Ramchandra rao's practical experience was taken. As per his practical observation, in the context of this formulation *Gandhaka Jarana* is said to be completed based on these following points**:**

1) The semisolid matter that was converted into granule form on adding *Swarasa* should remain as such even after the complete evaporation of the *Swarasa.*

2) When the *Shalaka* was introduced up to bottom of *Yantra* it should be felt as if it was kept in sandy gravel and when taken out no matter should adhere to it.

After completion of the pharmaceutical process, the final drug (GABR) was subjected to analysis through X-Ray Diffraction studies (XRD), Scanning Electron Microscope (SEM), Energy Dispersive X-Ray Analysis (EDX), Particle size analysis (PAS) and Zeta Potential (ZP).

MATERIALS AND METHODS: Materials -Double distilled Mercury and Crystals of Sulphur were obtained from local market of Tirupati, Fresh leaves of *Kakamachi, Tambula, and Meghanada* were collected from local market. Fresh leaves of *Dhatura* were collected from TTD's S. V. Ayurvedic College, Herbal garden, Tirupati. The drug GABR was prepared in Department of Rasa Shastra and Bhaishajya Kalpana, S. V. Ayurvedic College, TTD, Tirupati. Requirement for XRD: Powder X-Ray Diffractometer D8 Advanced, Manufacturer- BRUKER, Germany, SEM and EDX: Model- EVO MA 15, Manufacturer-Carl Zeiss, Germany, PAS: Microtrac Bluewave Particle Size Analyzer, Manufacturer -Nikkiso, Japan. ZP: Model-Malvern Zetasizer Nanao, Manufacturer- Malvern Instruments, UK.

XRD: The final product GABR was subjected to XRD at Department of Nuclear Physics, VIT University, Vellore.

Principle of XRD: X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation. Collimated to concentrate and directed towards the sample. The interaction of the incident rays with the sample produces constructive interferences (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda = 2d \sin\theta$). This law relates the wave length of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of

the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing allows identification of the mineral because each mineral has set o unique d- spacing. Typically, this is achieved by comparison of d-spacing with standard reference patterns.

Procedure: Sample is powdered in a agate mortar to very fine powder. It is mounted in a sample tray o machine. X-ray beam bearing a wavelength of 1.540598 A° from copper source is passed on to the sample. Detector was set to identify diffracted beams between 10-70 degrees of 2θ range. Obtained values are plotted on graph with the help of inbuilt "Reyflex Software" for further analysis.

SEM and EDX: The final product GABR was subjected to SEM and EDX at Department of Physics, S.V. University, Tirupati.

Preparation of SEM specimen: Specimen of the sample to be analyzed is directly kept on the specimen holder for visualization. As the sample employed has nonconductive nature, the sample surface is coated by carbon by arc melting technique.

Principle of EDX: The excess energy of the electron that migrates to an inner shell to fill the newly created hole can do more than emit an X-ray. Often, instead of X-ray, the excess energy is transferred to a third electron from a further outer shell, prompting its ejection. This ejected electron is called an Auger electron, and the method for its analysis is known as Auger electron spectroscopy (AES).

Procedure: Electron beam excitation is used in electron microscopes, Scanning electron microscopes (SEM) and Scanning transmission electron microscopes (STEM). 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 onto an analyzer for data display and analysis. The most common detector now is Si(Li) detector cooled to cryogenic temperatures with liquid nitrogen; however newer systems are often equipped with silicon drift detectors (SDD) with Peltier cooling systems. The detector used in the EDX is often the Lithium drifted Silicon detector. This detector must be operated at liquid nitrogen temperature, When an X-ray strikes the detector, and it will generate a photo electron within the body of the Si. As this photoelectron travels through the Si, it generates electron-hole pairs. The electron s and holes are attracted to the opposite ends of the detector with aid of a strong electric field. The size of the current pulse thus generated depends on the number of electro-hole pairs created, which in turn depends on the energy of the energy o the incoming X-ray. Thus, an X-ray spectrum can be acquired giving information on the elemental composition of the material under examination.

ZP: The final product GABR was subjected to ZP at Department of soil sciences, Agriculture University, Tirupati.

Principle of ZP: The most widely used technique for determining the ZP of colloidal-sized suspensions is particle electrophoresis or micro electrophoresis i.e. the movement of charged particles suspended in a liquid under the influence of an applied electric field. This offers the possibility of measuring the complete mobility spectrum. ZP is measured by applying an electric field across the dispersion. Particles within the dispersion with a ZP will migrate towards the electrode of opposite charge with a velocity proportional to the magnitude of the ZP. The Zetasizer Nano

series instrument uses micro-eletrophoresis and electrophoretic light scattering technology to measure ZP and eletrophoretic mobility and then applying the Henry equation. The elctrophoretic mobility is obtained by performing an electrophoresis experiment on the sample and measuring the velocity of the particles using Laser Doppler velocimetry (LVD).

Sample preparation: A 1% concentration of *Gandhakaajeernabaddho rasa* sample was prepared in distilled water. The particles were well dispersed before analysis.

Procedure: The sample is taken in a 1ml syringe and injected slowly into the capillary cell (cuvette) through the sample port. Care should be 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.The capillary cell is then placed into the sample holder of the zeta sizer instrument for analysis.

Particle size analyser: The final product GABR was subjected to Particle size analysis at Department of soil sciences, Agriculture University, Tirupati.

Principle: Particles, emulsions and molecules in suspension undergo Brownian motion. This is the motion induced by the bombardment by solvent molecules that themselves move due to their thermal energy. If the particles or molecules are illuminated with a laser, the intensity of the scattered light fluctuates at a rate that is dependent upon the size of the particles as smaller particles are kicked further by the solvent molecules and move more rapidly.This technique measures the diffusion of particles moving under Brownian motion and convert this to size and a size distribution using the Stokes-Einstein relationship.

Materials: 1. Microtrac Particle Size Analyzer

2) *Gandhakaajeernabaddho rasa* – 1 gm

Procedure: The sample was mixed in water and sonicated for ten 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.The scattered light is captured by a detector over the course of the analysis to determine the rate of diffusion (i.e. how fast the particles move within a system due to Brownian Motion) and thus the average Hydrodynamic particle size (referred to as the Z-Average) is calculated on an intensity weighted basis using the Stokes-Einstein equation. In simple terms, small particles move/diffuse more rapidly than larger particles.

OBSERVATIONS AND RESULTS: X-ray diffraction:Table No. 1: Showing the details of matching peaks of XRD data for *Gandhakaajeernabaddho rasa*

S. no	Element/Molecule	JCPDS Ref. No	2θ	Intensity	FWHM
1	Metacinnabar (HgS)	96-101-1369	26.49	1000	0.1998
			43.76	361.7	0.1998
			52.68	341.8	0.1998
2	Sulphur(S_8)	96-101-1161	23.4	755.7	0.1998
			27.78	378.9	0.1998

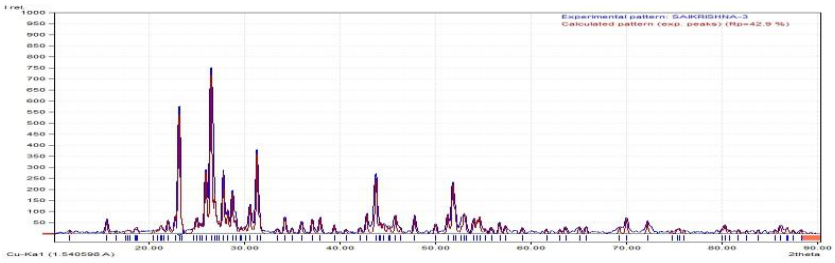


Fig. 1: Showing XRD graph of *Gandhakaajeernabaddho rasa*

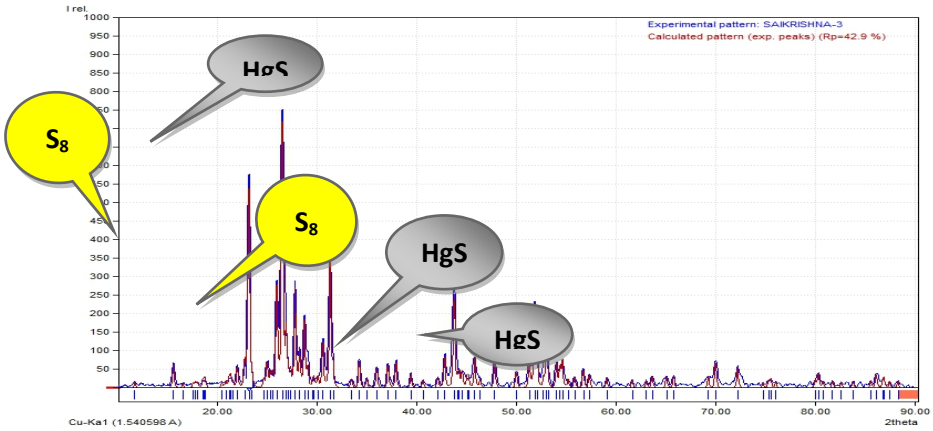


Fig. 2: Showing XRD graph of *Gandhakaajeernabaddho rasa*

Crystal details of JCPDS entries:

Entry # 96-101-1161

Phase classification

Name	Sulfur
Mineral Name	Sulfur
Formula	S_8
I/Ic	2.220000
Sample Name	1011160
Quality	C (calculated)

Crystal structure

Crystallographic data

Space group	F d dd (70)
Crystal system	Orthorhombic
Cell parameters	a= 10.4800 Å b= 12.9200 Å c= 24.5500 Å
Z	16

Atom coordinates

Element	Oxid.	*X*	*Y*	*Z*	Bi	Focc
S		-0.017	0.083	0.072	1.000000	1.000000
S		-0.094	0.161	0.200	1.000000	1.000000
S		-0.167	0.105	0.125	1.000000	1.000000
S		-0.094	0.028	0.250	1.000000	1.000000

Entry # 96-101-1369

Phase classification

Name	Mercury sulphide
Mineral Name	Metacinnabar
Formula	HgS
I/Ic	26.969999
Sample Name	1011368
Quality	C (calculated)

Crystal structure

Crystallographic data

Space group	F -4 3 m (216)
Crystal system	Cubic
Cell parameters	a= 5.8580 Å

Z	4						
Atom coordinates	**Element**	**Oxid.**	***x***	***Y***	***Z***	**Bi**	**Focc**
	Hg	2.0	0.000	0.000	0.000	1.000000	1.000000
	S	-2.0	0.250	0.250	0.250	1.000000	1.000000

XRD of GABR shows that, major peaks are of Hgs (Meta Cinnabar) compound with cubic structure and minor peaks are of S_8 (Sulphur) with orthorhombic structure. The HgS peaks are detected at diffraction angle of 26.49, 43.76 and 52.68. The JCPDS references number is 96-101-1369. S_8 (Sulphur) peak are detected at diffraction angle of 23.4 and 27.78. The JCPDS reference numberis 96-101-1161.

Scanning electron microscope: The images that were obtained from various regions of sample clearly depict that at different magnifications the grain size was commonly found to be ranging between 128nm at 5KX and 91nm at 7KX magnification**.** The bigger particles look like agglomeration of small particles.

Fig 3: Showing SEM result of *Gandhakaajeernabaddho rasa*(Mag. 5KX)

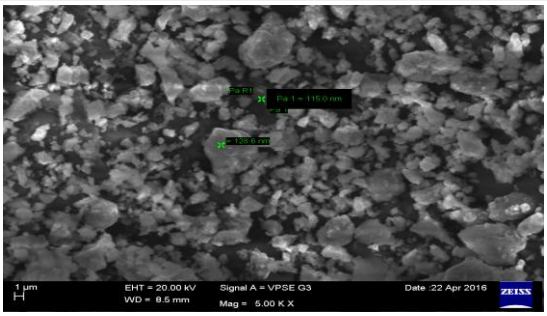
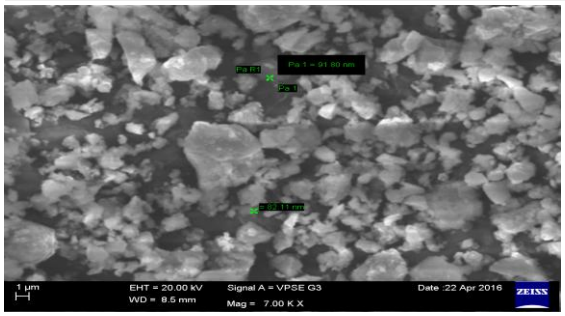


Fig. 4: Showing SEM result of *Gandhakaajeernabaddho rasa* (Mag. 7KX)



The average particle size detected through SEM was found to be **128nm at 5KX and 91nm at 7KX** magnitude.

Energy dispersive X-ray analysis

Element	**Weight %**	**Atomic %**
C	41.47	64.87
O	6.64	7.80
S	44.95	26.34
K	0.44	0.21
Hg	6.25	0.59
Total	100	100

Fig 5: Showing EDS graph of *Gandhakaajeernabaddho rasa*

EDS found that *Gandhakaajeernabaddho rasa* contains **Mercury 6.25%, Sulphur 44.95%, Carbon 41.75%, Oxygen 6.64% and Potassium 0.44%** by weight. **Zeta potential**

Table No.3:Showing Zeta potential measurement result of *Gandhakaajeernabaddho rasa*

Measurement Type	Zeta Potential
Sample Name	*Gandhakaajeernabaddho rasa*
Temperature of the holder	25^0C
Viscosity of the dispersion medium	0.895mPa-s
Conductivity	0.117mS/cm
Electrode Voltage	3.4V

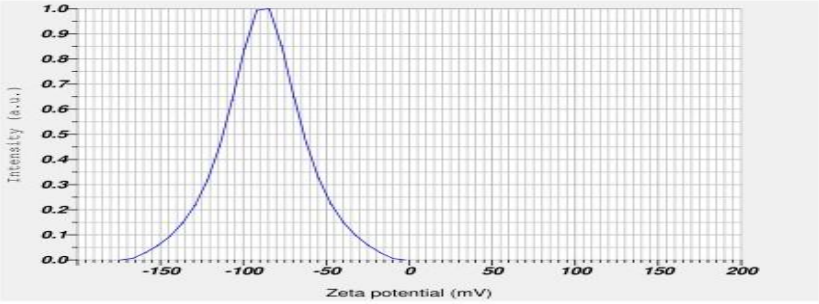


Fig. 6: Showing ZP measurment of *Gandhakaajeernabaddho rasa*

The Zeta Potential value of *Gandhakaajeernabaddho rasa* was found to be **-86.3mV** which indicates the colloidal stability.

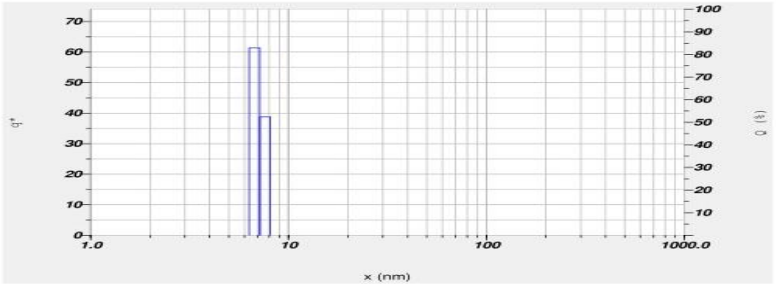
Particle size analyser Table No.4:Showing Particle size measurement results of *Gandhakaajeernabaddho rasa*

Measurement Type	Particle Size
Sample Name	*Gandhakaajeernabaddho rasa*
Scattering Angle	90
Temperature of the holder	25.2°c
T% before meas.	23211
Viscosity of the dispersion medium	0.892mPa.s
Form of Distribution	Standard
Representation of result	Scattering Light Intensity
Count rate	213kCPS

Peak No.	**S.P.Area Ratio**	**Mean**	**S.D.**	**Mode**
1	1.00	7.1nm	0.4nm	7nm
2	------	------nm	-----nm	------nm
3	------	------nm	-----nm	------nm
Total	1.00	7.1nm	0.4nm	7nm

Cumulant Operations:Z-Average:11933.1 nm,PI : 1.564

Fig. 7: Showing result of Particle size analysis of *Gandhakaajeernabaddho rasa*



The mean particle size of *Gandhakaajeernabaddho rasa* is **7.1 nm.**

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 provides us with 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 fingerprint charac-

terization 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, theirinter atomic distance and angle are. 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.XRD of *Gandhakaajeernabaddho rasa shows* that major peaks are of HgS (Meta Cinnabar) compound with cubic structure and minor peaks of Sulphur (S_8). The HgS peaks are detected at diffractive angle of 26.49, 43.76, 52.68 and sulphur peaks are detected at 23.4, 27.78. The JCPDS reference numbers are 96-101-1369 for HgS and 96-101-1161 for S_8.

Scanning electron microscopy (SEM) is an analytical technique that 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. It can magnify objects to extreme levels where even structure of nano particles could be clearly visible.Smallest particle size was found to be ranging between 1**28.0 nm at 5KX** magnification to **91 nm at 7KX** magnification. The bigger particles look like agglomeration of small particles. Smallest particle size proves that the drug can be easily absorbed in body and exhibit its therapeutic effect quickly.

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.This analysis confirmed thepresence ofelements viz. **C 41.47%, O - 6.64%, S - 44.95%, K - 0.44%, Hg - 6.25%.**

The size of the particles in the drug plays a major role in its therapeutic action and efficacy.The mean particle size of the particles in *Gandhakaajeernabaddho rasa* is 7**nm**with standard deviation of 0.47 nm. This nano size of the drug proves the fact that the Pharmaceutical processes adopted in the preparation of GABR might have reduced the particle size. The nano size of drug is indicative of its quick absorption and faster dispersion into body resulting into 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 *Gandhakaajeernabaddho rasa* found to be **-86.3mV**which indicates its high colloidal stability.

CONCLUSION: GABR was subjected to analysis with highly sensitive analyzers like XRD, SEM, EDS, ZP and PA for checking its identity, crystalline structure, particle size, absorption power and stability. XRD reports of *Gandhakaajeernabaddho rasa* shows the presence of Hgs (Metacinnabar) and Sulphur (S_8). In SEM the average crystalline size of the drug was between 91-121nm. In Particle size analysis the mean and mode values of the particle size in *Gandhakaajeernabaddho rasa* is 7nm with standard deviation of 0.47 nm

indicate quick absorption and faster dispersion into the body resulting in the better therapeutic efficacy. The Zeta Potential value of *Gandhakaajeernabaddho rasa* was -86.3mV which indicates the colloidal stability.

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Corresponding Author:
Dr.Kumar Saikrishna– Pg Scholar, Department Of Rasa Shastra And Bhaishajya Kalpana, S.V.Ayurveda College, T.T.D, Tirupati.
Email: saikrishna,osk.627@gmail.com