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ANALYTICAL STANDARDIZATION OF KANYALOHADI VATI

Dr. R. M. Sireesha*[1], Venkata Subbaiah K.[2] and Sridurga Ch.[3]

[1]PG Scholar Final Year, Department of Rasa Shastra and Bhaishajya Kalpana.

[2]Scientist, Department of Science and Technology, PURSE, Sri Venkateswara University.

[3]Professor and HOD, Department of Rasa Shastra and Bhaishajya Kalpana S. V. Ayurvedic College, Tirupati.

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***Corresponding Author**
Dr. R. M. Sireesha
PG Scholar Final Year,
Department of Rasa Shastra
and Bhaishajya Kalpana.

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. *Kanyalohadi Vati* is one such *Rasaoushadhi* mentioned in *Rasa Tantra Sara va Siddha Prayoga Sangraha* indicated in *Kashtartava*, which contains *Kaseesa bhasma* as one of the ingredient along with the herbal drugs like *Twak, Ela, Sunthi, Elua (Musambaram)* and *Gulkand*. *Shodhana, Marana, Elua nirmana, Gulkand nirmana* and *Churna Nirmana* are the main pharmaceutical procedures involved in the preparation of *Kanyalohadi Vati*. 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), 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 *Kanylohadi Vati* showed major peaks of Iron oxide (FeO) compound with Rhombohedral structure and Magnesium Iron Aluminium Oxide ($MgFeAlO_4$) with cubic structure. SEM micrographs showed presence of particles which are clusters of irregular shaped flakes at 500 X, 1.00 KX, 2.00 KX & 4.00 KX magnification. Particle size was found to be 10.7 nm with Zeta Potential of -10.4 mV. UV- Spectrum of *Kanylohadi Vati* showed maximum absorption at 230 nm and 283 nm. FTIR test showed 17 peaks between the wavelength 3856.34 cm^{-1} to 404.29 cm^{-1}. ICP–OES analysis revealed Iron and Potassium as the main constituent in 44122.23 ppm and 19640.55 ppm.

KEYWORDS: *Kanyalohadi vati*, Analytical studies, Safety.

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.

Kanyalohadi Vati is one of the important Herbo-mineral formulations mentioned in Rasa Tantra Sara va Siddha Prayoga Sangraha - Prathama Khanda –Gutika Prakarana.[1]

Table 1: Showing contents of *Kanyalohadi vati.*

S. No.	Name of content	Quantity
1	*Elua (Musambaram)*	10 parts
2	*Kaseesa bhasma*	7 parts
3	*Twak*	5 parts
4	*Ela*	5 parts
5	*Sunthi*	5 parts
6	*Gulkand*	20 parts

All the *Dravyas* possess various therapeutic properties indicated in the management of several diseases. Classical texts have enumerated certain tests which indicate the proper transformation of basic metal into bio-absorbable *Bhasma* form. In spite of these facts, *Bhasmas* of metals are always under debate, not only in sense of its therapeutic excellence also for unnecessary hue and cry about their toxicity and safety. Therefore modern analytical techniques are expected to help in circumventing this problem. Hence highly sensitive modern parameters like X-Ray Diffraction, Scanning Electron microscopy, 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

- *Kaseesa* was collected from Chennai, *Kumari* leaves were collected from Herbal garden S.V Ayurvedic Pharmacy, Srinivasa Mangapuram, TTD, Tirupati. Rose petals, *Twak, Ela*

and Sunthi were obtained from local market, Tirupati. Entire preparation of *Kanylohadi Vati* was carried out in Department of Rasa Shastra and Bhaishajya Kalpana, TTD's S.V.Ayurvedic College and Sri Srinivasa Ayurvedic Pharmacy, Srinivasa Mangapuram, Tirupati.

- Requirement for XRD: Model- Powder X-Ray 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 Spectro-photometer, Manufacturer- Thermo Scientific, India; ICP-OES: Model- Agilent 725, Manufacturer- Agilent technologies, USA.

Pharmaceutical process

The pharmaceutical procedures adopted in this study are *Shodhana, Bhavana, Marana, Elua nirmana, Gulkand nirmana, Churna nirmana* and preparation of *Kanyalohadi Vati*. *Shodana* of *Kaseesa* was done by triturating with *Bringaraja Swarasa* for 3 times.[2] *Shodhita Kaseesa* was taken in a *Khalva yanra* and triturated with *Nimbu swarasa. Chakrikas* of uniform size were prepared and placed in a *Sharava* and subjected to *Sharava samputikarana*. This was subjected to *Laghu puta.*[3] The entire procedure was performed for 6 times. Then *Kaseesa bhasma* having all the *Bhasma lakshnas* was obtained. *Elua* was prepared by heating *Kumari swarasa* over moderate flame untill it gets turned into semisolid consistency.[4] *Gulkand* was prepared by using Rose petals and Sugar.[5] Raw drugs of *Twak, Ela* and *Sunthi* were made into fine powder.[6] Then 7 parts of *Kaseesa bhasma,* 10 parts of *Elua,* 20 parts of *Gulkand* and 5 parts each of *Twak churna, Ela churna* and *Sunthi churna* were mixed together to prepare *Kanyalohadi Vati* of 500 mg.

Analysis of *Kaseesa Bhasma* using ancient parameters (*Bhasma Pariksha*)

The final *Bhasma* was analyzed for quality control as described in the ancient texts and the following observations were made:

***Rekhapurnatva*[7]:** After proper trituration, small amount of *Bhasma* was taken between thumb and index finger. It filled into the fine lines of fingers. *Rekhapurnatwa* was obtained after 4th *Puta*.

***Varitaratwa*[8]:** 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 5th *Puta.*

Nischandratwa: Small quantity of *Bhasma* was observed under bright sunlight for presence of any free shiny metal particle.

Niswadu Pareeksha: When a small amount of the *Bhasma* was kept on tongue, there was not any feeling of taste / untoward sensation.

***Dantagrenakachkachabhavati*:** When a small amount of the *Bhasma* was placed between the teeth, no sandy feeling was appreciated.

***Anjanasadrishyasukshmatva*:** The *Bhasma* prepared was fine like collyrium.

***Avami*:** Ingestion of small amount of the *Bhasma* did not produce any nausea / vomiting.

Laghutva (lightness), *Amlatva* and *Mrudutwa* were attained after the whole process.

Kaseesa turned from Blackish grey to Blackish brown, dark brown, and then to red (*Sindhura*) by the end of 6th *puta*.

Analysis of *Kanylohadi Vati* using modern parameters X-Ray Diffraction (XRD)

Kanylohadi Vati 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 (SEM)

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

Procedure: 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 using arc melting technique. Then the dried powder was observed under the

microscope at 500 X, 1.00 KX, 2.00 KX & 4.00 KX magnification and the micrographs were taken with the inbuilt camera.

Particle Size Analysis

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.

Zeta Potential

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

Procedure of ZP: A 1% concentration of *Kanylohadi Vati* 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 *Kanylohadi Vati* 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 *Kanylohadi Vati* was taken in Teflon tubes and added to 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 PaarMultiwave 3000). Then the *Kanylohadi Vati* 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 *Kanyalohadi Vati.*

S. No	Element/Molecule	JCPDS Ref. No	2θ	Intensity	FWHM	h k l
1.	**FeO (Iron Oxide)**	89-0690	35.71	178	0.216	0 0 6
2.	**FeS (Iron Sulfide)**	89-6928	29.21 30.84 31.32 36.19 41.32 43.91 54.71 57.82 64.55	1101.35 286.24 232.72 1080.38 50.81 157.79 131.74 185.80 153.80	0.168 0.192 0.288 0.192 1.152 0.336 0.384 0.384 0.384	1 0 3 1 1 0 0 0 4 0 1 8 0 1 11 1 1 4 0 2 0 2 0 3 2 2 2
3.	**$MgFeAlO_4$ (Magnesium Iron Aluminium Oxide)**	01-073-2159	45.79	23	0.288	-2 0 4
4.	**Fe_4KS_5 (Potasssium Iron Sulfide)**	83-1824	14.97 19.59 24.69 26.46 28.57 33.72 50.01 63.46	70.74 108.52 137.62 222.16 390.34 307.49 132.79 227.94	0.240 0.192 0.576 0.288 0.192 0.288 0.288 0.384	1 1 2 2 1 1 2 0 4 1 3 0 1 1 6 3 0 5 4 4 2 5 3 8

Table 2: Showing Crystal details of JCPDS entries Entry # 89-0690 Crystal Structure.

Space group	**R3(148)**
Crystal System	**Rhomboherdal**
Cell Parameters	**a = 6.070726(3) A° b= 15.094757(3) A° c = 2.4865 A°**
Z	**24**

Entry # 00-021-0925

Crystal Structure

Space group	**A2/a**
Crystal System	**Monoclinic**
Cell Parameters	**a = 7.6240 A° b= 7.4680 A° c = 7.1230 A°**
Z	**4.0**

Entry # 01-073-2159

Crystal Structure

Space group	**Fd-3m**
Crystal System	**Cubic**
Cell Parameters	**a = 8.2240 A° b=8.2240 A° c =8.2240 A°**
Z	**8.00**

Entry # 83-1824

Crystal Structure

Space group	**14/mmm(139)**
Crystal System	**Tetragonal**
Cell Parameters	**a =10.424 A° b= 20.626 A° c = 1.9787 A°**
Z	**2.0**

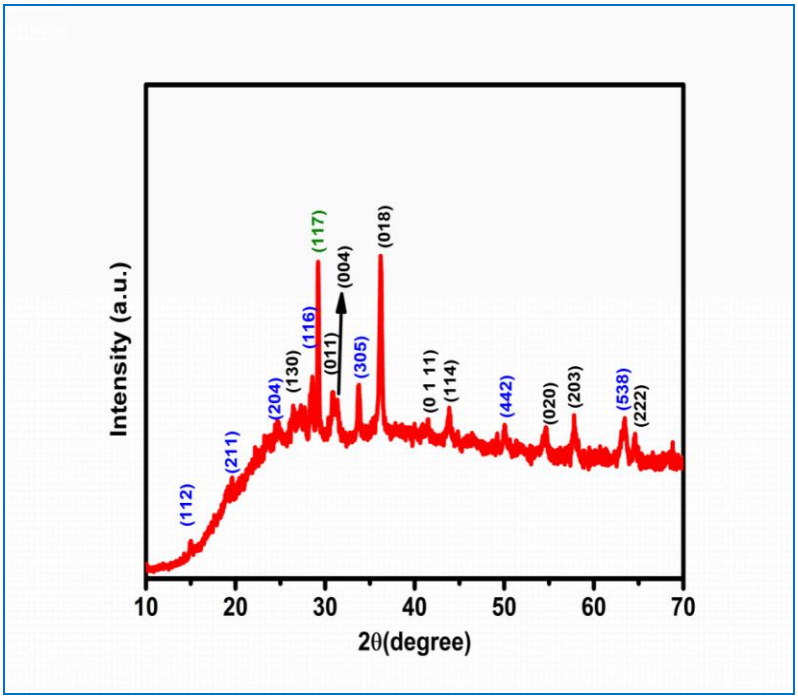

Figure 1: Showing XRD graph of *Kanyalohadi Vati*.

XRD of *Kanyalohadi Vati* shows that major peaks are of Iron oxide (FeO) compound with Rhombohedral structure and Magnesium Iron Aluminium Oxide ($MgFeAlO_4$) with cubic structure. The FeO peaks are detected at diffractive angle of 35.65 and 46.86, $MgFeAlO_4$ peaks is detected at 54.62. The JCPDS reference numbers are 890690 for FeO, 01-073-2159 for $MgFeAlO_4$.

Scanning Electron Microscopy

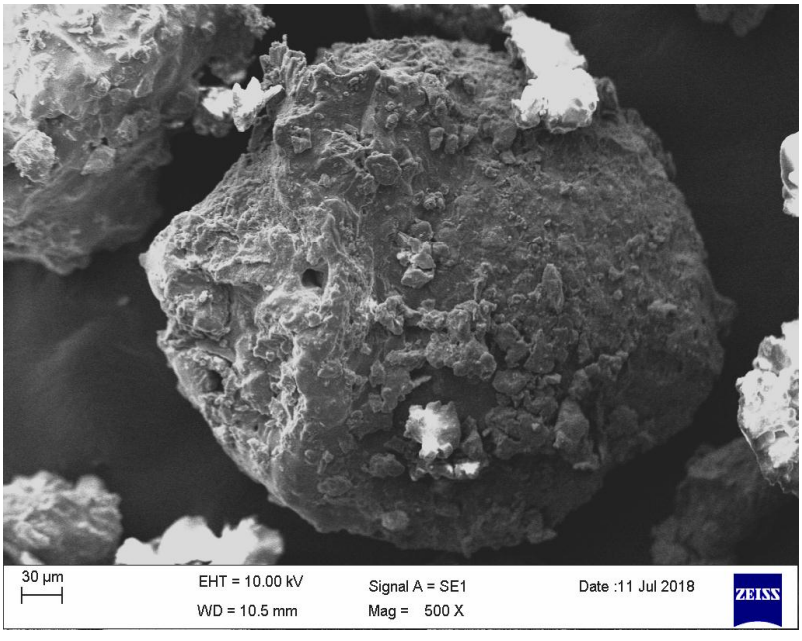

Figure 2: Showing SEM result of *Kanyalohadi Vati* (Mag. 500 X).

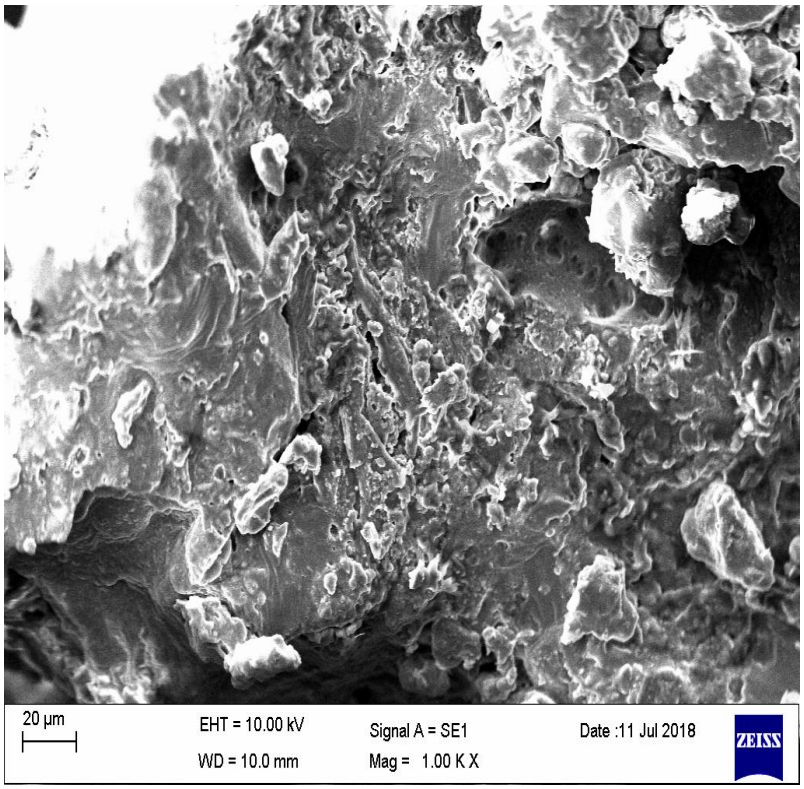

Figure 3: Showing SEM result of *Kanyalohadi Vati* (Mag.1.00 KX).

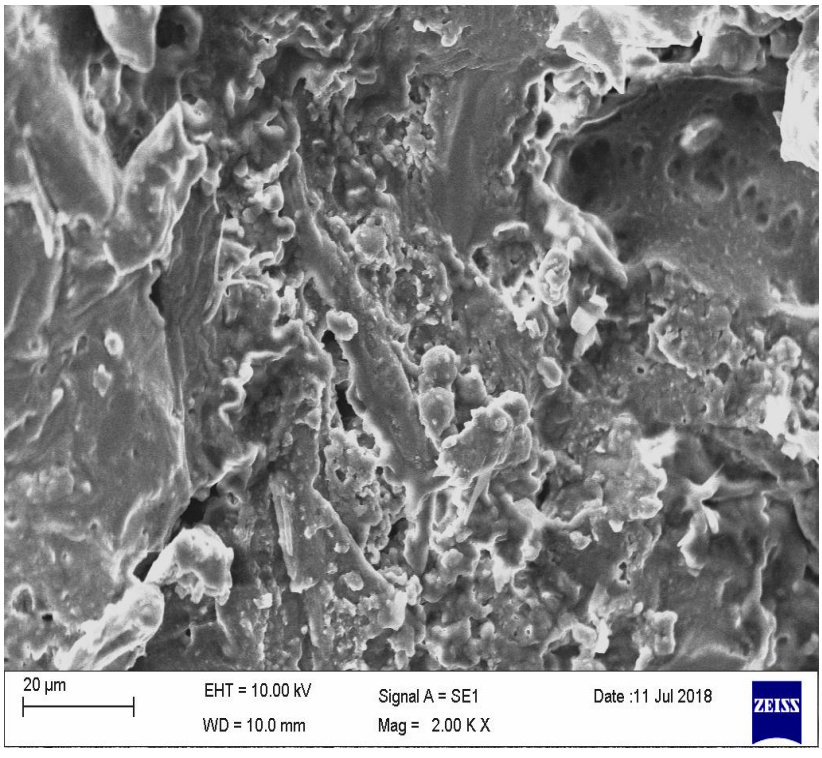

Figure 4: Showing SEM result of *Kanyalohadi Vati* (Mag. 2.00 KX).

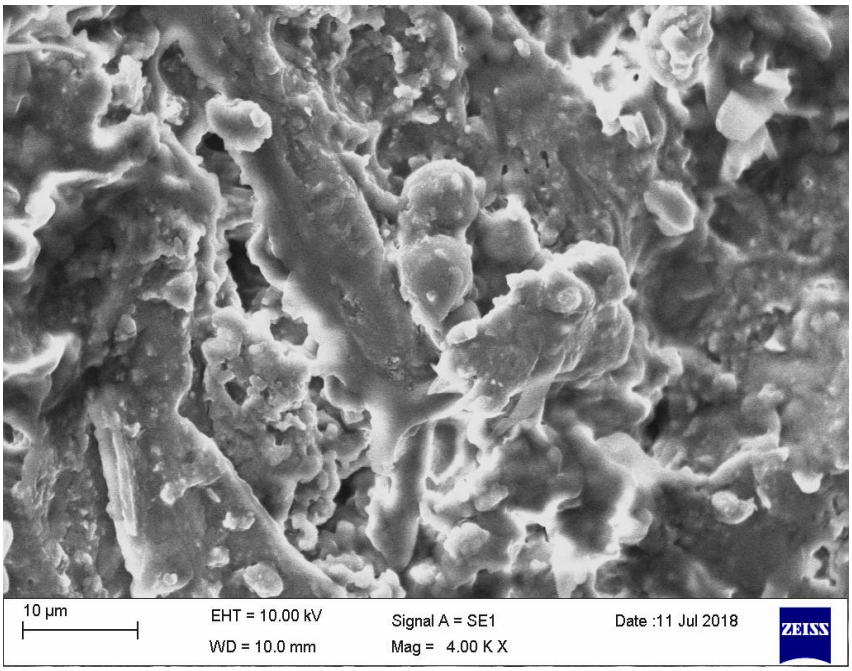

Figure 5: Showing SEM result of *Kanyalohadi Vati* (Mag. 4.00 KX).

SEM analysis of *Kanyalohadi Vati* showed the presence of particles which are clusters of irregular shaped flakes at 500 X, 1.00 KX, 2.00 KX & 4.00 KX magnifications.

Particle Size Analysis

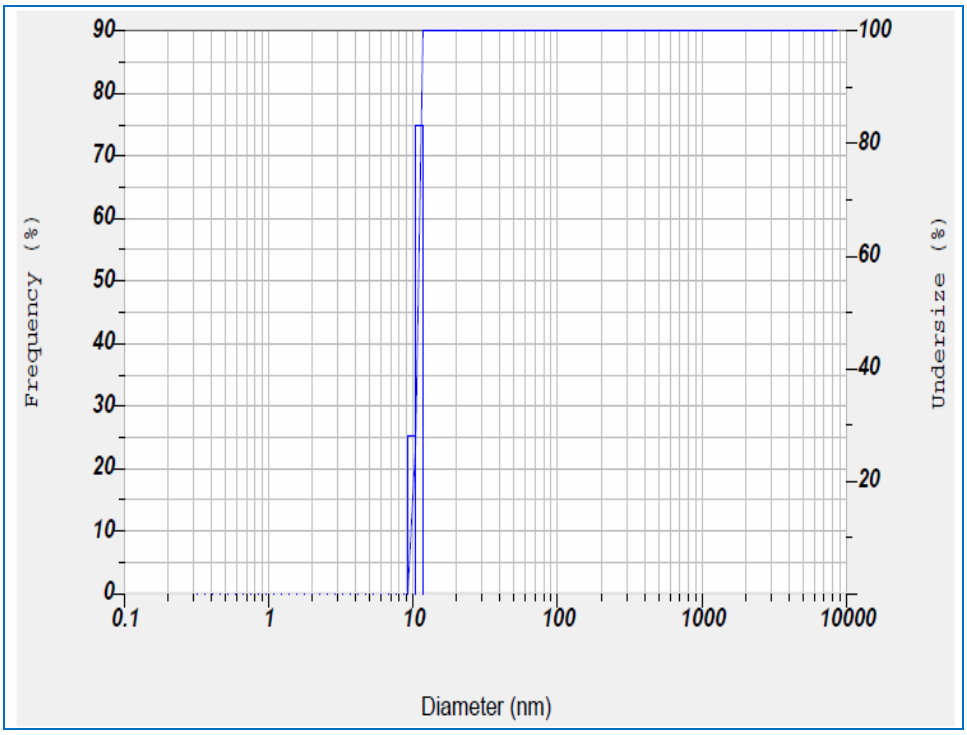

Figure 6: Showing the result of Particle size analysis of *Kanyalohadi Vati.*

The mean particle size of *Kanyalohadi Vati* is **10.7 nm.**

Zeta Potential

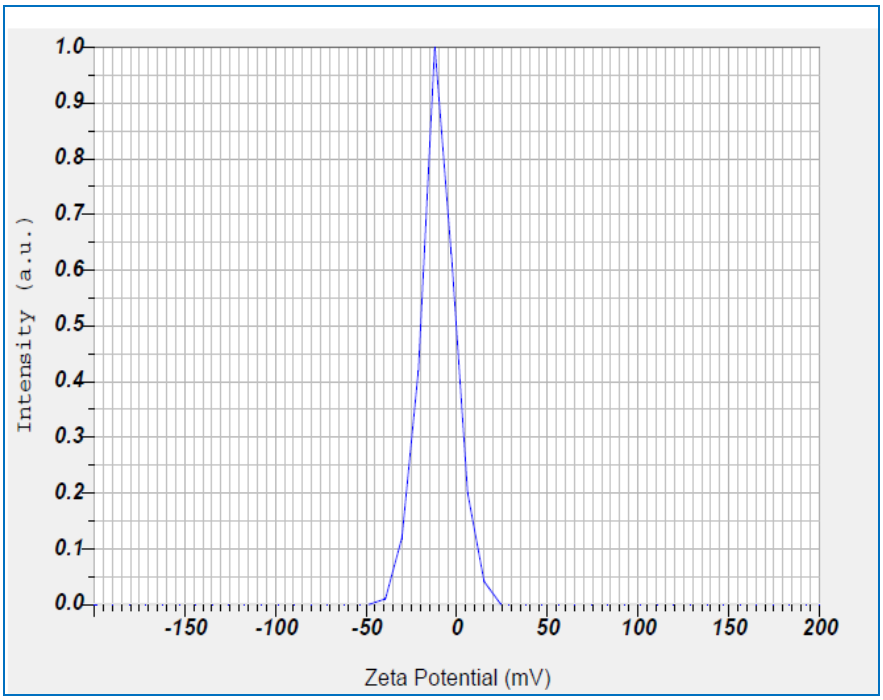

Figure 7: Showing Zeta potential distribution of *Kanyalohadi Vati.*

Kanyalohadi Vati **sample showed a Zeta potential value of -10.4 mV, which indicates moderate colloidal stability.**

UV- Spectroscopy

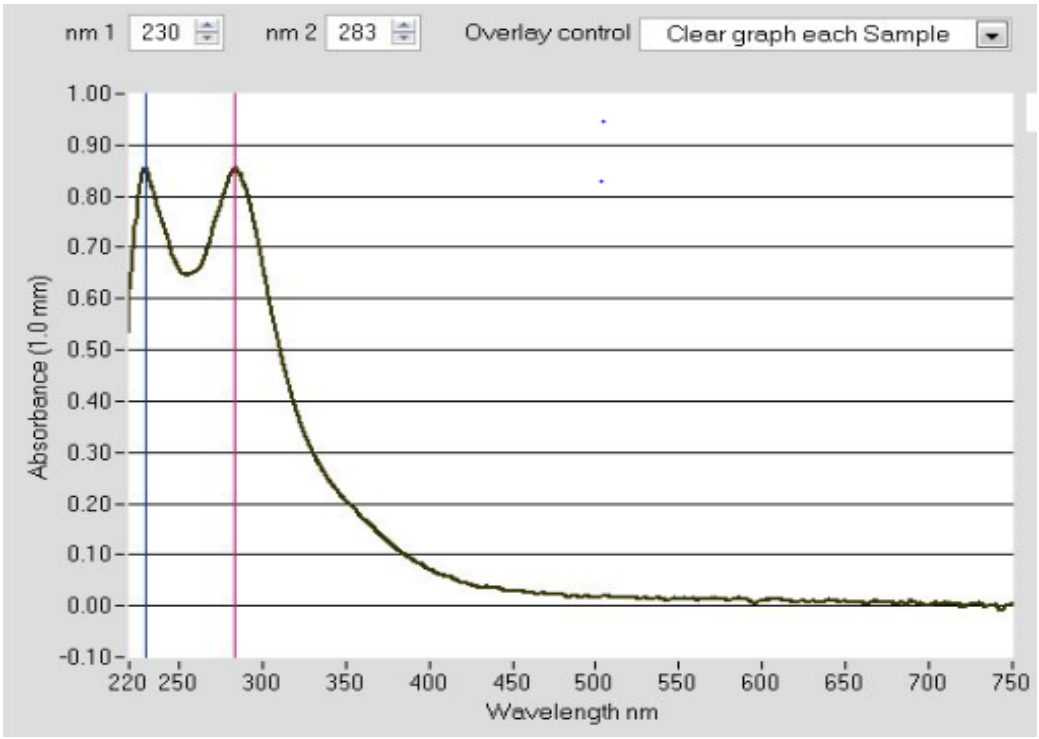

Figure 8: Showing UV-Spectrum (UV-light) of *Kanyalohadi Vati.*

UV-Spectrum of *Kanyalohadi Vati* Showed Maximum absorption at 230 nm and 283 nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

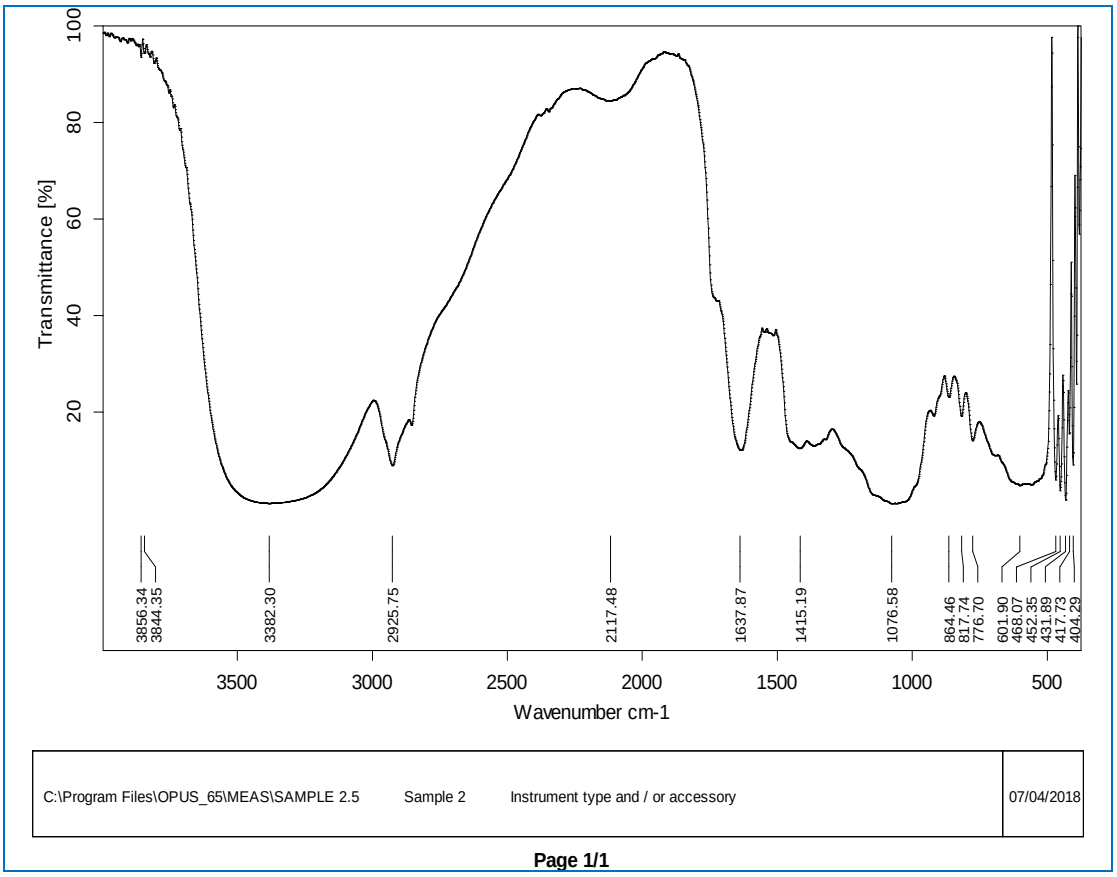

Figure 8: Showing various peaks obtained in FTIR analysis of *Kanyalohadi Vati.*

Table 2: Showing details of Peaks obtained in FTIR analysis of *Kanylohadi Vati.*

Sample Name	No. of Peaks	Wavelength
Kanyalohadi Vati	17	3856.34, 3844.35, 3382.30, 2925.75, 2117.48, 1637.87, 1415.19, 1076.58, 864.46, 817.74, 776.70, 601.90, 468.07, 452.35, 431.89, 417.73, 404.29

Table 3: Various peaks obtained in FTIR analysis of *Kanyalohadi Vati* and their correlation with compounds.

S. No.	Actual peak	Bond	Type of bond	Appearance
1.	3856.34	-	-	Not found
2.	3844.35	-	-	Not found
3.	3382.30	O-H	Alcohols	High concentration broad
4.	2925.75	C-H	Alkanes	Strong
6.	2117.48	C≡C	Alkyne	Moderate Weak
7.	1637.87	C=C	Alkene	Moderate Weak
8.	1415.19	C-H	Alkanes(-CH_3)bend	Moderate
9	1076.58	C-O	Alcohols, Ethers, Esters, Carboxylic Acids, Anhydrides	Strong
10.	864.46	C-H	Aromatics	Strong
11.	776.70	C-H	Aromatics	Strong
12.	601.90	-	-	Not found
13.	468.07	-	-	Not found
14.	452.35	-	-	Not found
15.	431.89	-	-	Not found
16.	417.73	-	-	Not found
17.	404.29	-	-	Not found

Inductively Coupled Plasma – optical Emission Spectrometry

Table 4: Showing the result of ICP-OES analysis of *Kanyalohadi vati*.

S. No.	Name of the elements analysed	Tests results in ppm
1.	Arsenic	Not detected
2.	Aluminium	828.37
3.	Barium	66.38
4.	Beryllium	Not detected
5.	Bismuth	101.01
6.	Calcium	7579.99
7.	Cadmium	4.67
8.	Chromium	61.29
9.	Copper	27.62
10.	Cobalt	5.38
11.	Iron	44122.23
12.	Potassium	19640.55
13.	Magnesium	3075.12
14.	Manganese	371.72
15.	Nickel	124.88
16.	Sodium	677.40
17.	Lead	14.67
18.	Selenium	Not detected
19.	Silver	22.98
20.	Sulphur	15453.20
21.	Strontium	50.26
22.	Vanadium	1.35
23.	Zinc	424.89

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 research 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.

The *Kaseesa Bhasma* passed all *Bhasma Parikshas* viz. *Varitaratva, Rekhapurnatva, Nischandratva and Slakshnatva* which proves that drug has attained its *Bhasma* form properly which proves that neither less nor more heat is desirable and *'Supaka'* is essential for making a drug safe and efficacious.

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 material 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 *Kanyalohadi Vati* shows that major peaks are of Iron oxide (FeO) compound with Rhombohedral structure and Magnesium Iron Aluminium Oxide($MgFeAlO_4$) with cubic structure. In *Kanyalohadi Vati*, along with Iron Oxide there were peaks of Magnesium Iron Aluminium Oxide. This may be due to presence of these elements in herbal ingredients of *Kanyalohadi Vati* which forms new compounds.

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 *Kanyalohadi Vati* as clusters of irregular shaped flakes may be due to accumulation of herbal drugs over the surface of *Kaseesa Bhasma.*

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 *Kanyalohadi Vati* is 10.7 nm. 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 *Kanyalohadi Vati* was found to be -10.4 mV which indicates moderate colloidal stability. High zeta potential indicates easy dispersion, whereas less zeta potential indicates strong aggregation of particles in suspension.

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 *Kanyalohadi Vati* showed maximum absorption at 230 nm and 283 nm.

FTIR was performed to detect the presence of functional groups or organic legends in *Kanyalohadi Vati*. 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. *Kanyalohadi Vati* showed 17 peaks between the wavelength 3856.34 cm^{-1} to 404.29 $cm^{-1.}$ FTIR analysis of *Kanyalohadi Vati* reveals the presence of functional groups O-H, C-H, C≡C, C=C, C-O i.e., alcohols, alkanes, alkyne, alkene and aromatic groups.

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 *Kanyalohadi Vati* reveals the following elements (in PPM units) i.e. Iron-44122.23, Potassium-19640.55, Sulphur- 15453.20, Calcium-7579.99, Magnesium-3075.12, Aluminium-828.37, Sodium-677.40, Zinc-424.89, Manganese-371.72 etc. Cinnamom zeylanica, Eletteria cardomum and Zingiber officinalis[9] contains the following elements i.e., Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium, Zinc, Copper and Manganese. The heavy metals like Selenium and Arsenic were not detected in the sample.

CONCLUSION

The present study confirms the fact that *Kanyalohadi Vati* 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 and from the component drugs of *Kanyalohadi Vati*. These entire analytical tests justify the structural and chemical composition of the compound indicating its safety and efficacy.

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