Research Article

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ANALYTICAL STUDY OF SHANKHAPANI RASA: A HERBOMINERAL COMPOUND

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ABSTRACT

Shankhapani Rasa is a Herbo-Mineral formulation which is effective in the management of Kashtartava (Dysmenorrhoea), was mentioned in Gutika Yoga Prakaranam of Sahasrayogam. It is prepared with Suddha Parada *(*Mercury*)*, Suddha Gandhaka *(*Sulphur*)*, Shodhita Vatsanabhachurna *(Aconitum ferox* wall.ex seringe*)*, Hinguchurna *(Ferula foetida,* Regel*)*, Saindhava lavana *(*Rock Salt*)*, Chincha Ksara *(Tamarindus.indica)*, Shankha Bhasma, Trikatu churna *(Zingiber officinale* Roxb*, Piper longum, Piper nigrum)*. Pharmaceutically prepared Shankhapani Rasa was subjected for organoleptic tests, Physico-chemical analysis, X-Ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDAX) with a view to standardize the drug through modern techniques. This article highlights the procedures and techniques adopted for the physico-chemical analysis and characterization of the Shankhapani Rasa. XRD of Shankhpani Rasa has shown major phases of HgS (Meta cinnabar), S, and S_8 peaks. The quantity of elements analysed in SEM with EDAX are Hg-4.52%, Ca-12.79%,Cl-14.11%,Na-8.84% and K-9.77%.

Key Words: Shankhapani Rasa, Kashtartava, XRD, SEM-EDAX.

INTRODUCTION

Rasa Shastra has a glorious past regarding Good Manufacturing Practices (G.M.P.) Standardization, Standard Operating Procedure (S.O.P) etc. Now a day's commercialization in the field of Ayurvedic pharmacy resulted in increased urge for making surplus amount for profit, which has promoted use of substandard raw materials and manipulation in S.O.Ps' of Ayurvedic drugs. Thus, in recent times Ayurveda has obstacles in its way to provide quality treatment because of the unavailability of safe and efficacious drug. As the efficacy, safety and authenticity of various Herbo-Mineral formulations still remains a big question, there is a need for proper standardization of Ayurvedic drugs at various levels starting from the selection and collection of raw material to the final product. Analytical study is the key part of any scientific research. It tells about the correlation between pre-determined hypothetical values and actual results obtained. It gives valuable information about safety, efficacy, stability etc. of any formulation.

MATERIALS AND METHODS

Shankhapani Rasa[1] was prepared with Suddha Parada[2], Suddha Gandhaka[3], Shodhita Vatsanabhachurna[4], Hinguchurna, Saindhava lavana, Chincha Ksara[5], Shankha Bhasma[6], Trikatu churna in the department of Rasashastra, S.V. Ayurvedic college, Tirupati. Analytical Study was carried out under different sections, namely Organoleptic tests (colour, odour, taste, touch, appearance),Physico-Chemical tests (Moisturevalue, pHvalue, Total ash value, Acid insoluble ash value, Water Soluble ash value), X-Ray Diffraction (XRD), Scanning Electron Microscope Studies (SEM) with EDAX..

Organoleptic tests

It helps in providing basic information about drugs. This generally includes tests that can be done by one's sensory organs and quality of material can be inferred up to some extent.

Physico-Chemical tests

Physico-Chemical tests were conducted at Department of Home Science, S. V. University, Tirupati, India.

Determination of pH value

The pH meter and electrode system was operated according to the manual instructions. At the end of a set of measurements, reading of the solution used for standardizing the meter and electrodes was taken. This reading should not differ by more than 0.02 from the original value at which the apparatus was standardized. Then 1 gram of Shankhapani Rasa sample was put in 5 ml. of water and pH was determined for the solution[7]. The obtained pH value of Shankhapani Rasa is 7.3.

Determination of Moisture Value

The digital balance is calibrated to 0.00gms. Sample is weighed on balance to check accuracy of weight and is taken in a porcelain crucible. Hot air oven thermostat is adjusted to 105°C and left for certain time to get stabilized at that temperature. Porcelain crucible with sample is kept on oven tray with equidistant from four walls of oven. Sample is dried for one

hour. Porcelain crucible is taken out and kept in desiccator to prevent any moisture absorption. After self-cooling porcelain crucible with sample is weighed to calculate the loss of weight on drying. The percentage content of moisture value is calculated in percentage (%w/w). Similar procedure is repeated for three times. Arithmetic mean is calculated from the values of three attempts to obtain the probable best accurate value.

Determination of Total Ash Value

Two grams of accurately weighed sample was taken and transferred to the cleaned, dried, and weighed silica crucible and was subjected to ignition using electric furnace at 450^0 C for an hour. Silica crucible was taken out from the furnace and was allowed to cool and weighed. After cooling the weight of the ash was obtained and the ash value of sample was calculated[8]. Similar procedure was repeated for three times. Arithmetic mean was calculated from the values of three attempts to obtain the probable best accurate value.

Determination of Acid Insoluble Ash Value

2 g of sample was digested with 25 ml diluted hydrochloric acid for 5 minutes, then filtered through Whatman paper and washed with water. The residue was taken in a crucible dried and ignited, allowed to cool and weighed[8]. Similar procedure is repeated for three times. Arithmetic mean is calculated from the values of three attempts to obtain the probable best accurate value.

Determination of Water Soluble Ash Value

The digital balance is calibrated to 0.00gms. Sample is weighed on balance to check accuracy of weight. Further using 25 ml. of double distilled water, total ash is washed into a 100 ml beaker. Beaker is boiled for 5 minutes. Filtered through an ash less filter paper, residue is washed twice with hot water. Filter paper placed in a silica crucible then, incinerated by gradually increasing the heat in a muffle furnace at 450º C for some hours. After complete incineration, it is kept in a desiccator to cool. The weight of ash with silica crucible is noted. Then the loss of ash in water is calculated and water soluble ash value is quantified in terms of percentage (% w/w). Similar procedure is repeated for three times. Arithmetic mean is calculated from the values of three attempts to obtain the probable best accurate value.

X- Ray Diffraction Studies

X-ray diffraction has been in use in two main areas, for the fingerprint 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 are 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.

Instrument: Diffractometer System XRD – 3003 TT model, manufacturer General Electronics – United States

Place of study: Department of Nuclear Physics, Dept. of Madras, Guindi Campus, Chennai, India

Analysis Procedure

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

Scanning Electron Microscope Studies

The Scanning Electron Microscope (SEM) is a microscope that uses electrons rather than light to form an image. There are many advantages to using the SEM instead of a light microscope. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. Preparation of the samples is relatively easy since most SEMs only require the sample to be conductive. The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the most heavily used instruments in research areas today.

To produce the SEM image, the electron beam is swept across the area being inspected, producing many such signals. These signals are then amplified, analyzed, and translated into images of the topography being inspected. Finally, the image is shown on a CRT.

Instrument: EVO MA 15, Carl Zeiss-Germany

Place of study: Department of Physics, S.V. University, Tirupathi, India

Analysis Procedure: In this study the dried powder was placed over the specimen holder and observed under the microscope at 1,000X to 7,000X. Micrographs were taken with the inbuilt camera.

Observation: The Scanning Electron Microscopy (SEM) of Shankhapani Rasa at different magnifications, the grain size was commonly found to be ranging between 109.2nm at 5K magnification to 77.97nm at 7K. The smallest grain size is found to be 77.97nm at magnification of 7K in Shankhapani Rasa sample. Particles are found to be agglomerated due to presence of natural resins which have partial binding nature. The bigger particles look like agglomeration of small particles**.**

Energy-dispersive X-ray Spectroscopy Analysis (EDAX)

A Scanning Electron Microscopy (SEM) may be equipped with an EDAX analysis system to enable it to perform compositional analysis on specimens. EDAX analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen.

Place of study: Department of Physics, S.V. University, Tirupathi, India

DISCUSSION

Shankhapani Rasa is brownish black in colour, Salty taste, odour of Hingu, and smooth to touch. It had no sound on chewing (shabda pareeksha), smooth to touch (sparsha pareeksha). Presence of no sound and touch indicates its smoothness, softness and fineness. The fineness was also observed in it with rekhapurnatwa. Moisture value determines the quantity of moisture a sample contains. The pH value is found to be 7.3, slightly alkaline nature due to presence of kshara dravyas cincha kshara, and shankha bhasma. The presence of moisture was due to absorption of moisture from surroundings. The level of moisture must not be high otherwise; it can reduce the shelf life by microbial growth. Shankhapani Rasa was found to have an average moisture content of 8.63% and an average total ash value of 66.36%.

Table 1: Organoleptic tests of Shankhapani Rasa

Parameter	Observation
Colour	Brownish black
Taste	Salty
Odour	Hingu
Touch	Smooth

Table 2: Physico-chemical analysis of Shankhapani Rasa

Test name	Values in % w/w			Arithmetic Mean of three attempts
	1st time	2nd time	3rd time	
Moisture value	8.66	8.60	8.65	8.63% w/w
Total Ash value	66.6	66.50	66	66.36% w/w
Acid insoluble ash value	17.03	17.54	17.90	17.22% w/w
Water soluble ash value	41.76	41.24	41.90	41.60% w/w

Table 3: Matching peaks of XRD data for Shankhapani Rasa

Element/Molecule	JCPDS Ref.No	2θ	Intensity	FWHM
S_8	01-078-1889	28.71	894.9	0.0799
S	01-089-6764	29.72	824.9	0.0799
HgS	00-019-0798	31.94	1000	0.0799
	00-042-1408	45.75	564.1	0.0799
	00-042-1408	47.81	252.1	0.0799
	00-006-0256	66.70	171.5	0.7581
$CaCo_3$	01-070-0095	36.27	159.9	0.0799
	01-070-0095	39.76	181.2	0.0799
KNO_2	01-073-1841	26.65	330.5	0.0799
KCl	00-001-0790	40.87	426.5	0.0799
KNO_3	01-071-1558	48.76	238.3	0.0799

Table 4: Compositions of Shankhapani Rasa with elemental % by EDAX

Element	Weight %	Atomic%
O	46.23	65.97
Na	8.84	8.98
S	3.74	2.66
Cl	14.11	9.09
K	9.77	5.70
Ca	12.79	7.29
Hg	4.52	0.51
Total	100.00	100.00

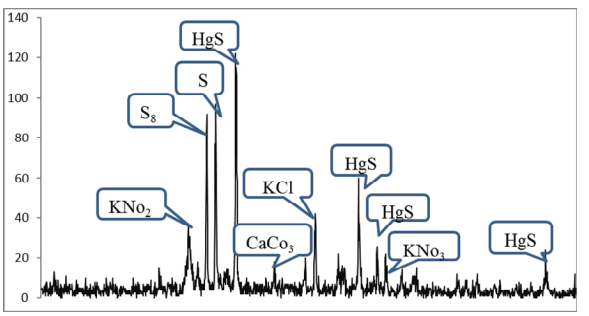

Figure 1:X-RD Pattern of Shankhapani Rasa

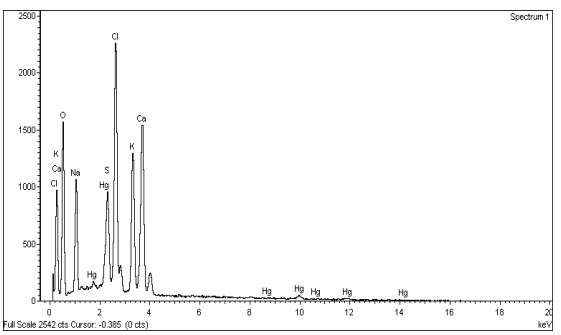

Figure 2: SEM picture of Shankhapani Rasa

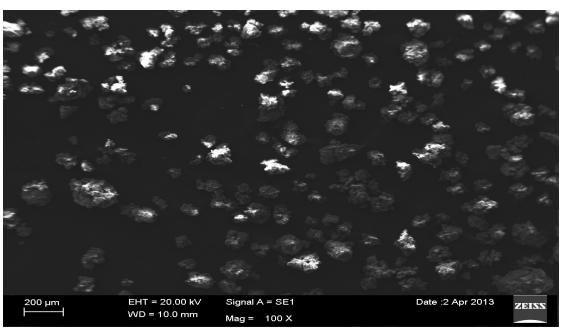

Figure 3: EDAX graph of Shankhapani Rasa

Shankhapani Rasa was analysed for solubility in water also and an average value was found to be 41.60% whereas Acid insoluble value was found to be 17.22%. Hence, it can be said looking at the results of acid in soluble ash value and water soluble ash value that, Shankhapani Rasa is more soluble in water.

In XRD study it has major phase HgS (Meta cinnabar), S and S_8 peaks. The HgS peaks are detected at diffraction angle of 31.94, 45.75 and 47.81. The JCPDS reference numbers are 00-019-0798 and 00-042-1408 respectively. Sulphur peaks are detected at diffraction angle of 28.71 and 29.72 the JCPDS reference numbers are 01-078-1889 and 01-089-6764 respectively. In minor phase it has $CaCo_3$,KNO_2, KCl, KNo_3 peaks detected at diffraction angle of 36.27, 26.65, 40.87 and 48.76.The JCPDS

reference numbers are 01-070-0095,01-073-1814,00-001-0790 and 01-071-1558 respectively. The shape of the crystals is monoclinic for S and orthorhombic for S_8. The presence of free sulphur can be attributed to the calculations, which were made basing on Stoichiometry. The major peaks formed were sharp due to crystalline nature of HgS, S and S_8. The other peaks like $CaCo_3$, KNo_2, KCl, KNo_3 formed were not sharp as those of mercury sulphide, and sulphur.

The Scanning Electron Microscopy (SEM) of shankhapani Rasa at different magnifications, the grain size was commonly found to be ranging between 109.2nm at 5K magnification to 77.97nm at 7K. The smallest grain size is found to be 77.97nm at magnification of 7K in Shankhapani Rasa sample. The smallest crystalline grain may be of Shankha bhasma in Shankhapani Rasa sample, the size of the particle might have reduced because of trituration. Particles are found to be agglomerated due to presence of natural resins which have partial binding nature. The bigger particles look like agglomeration of small particles. SEM with EDAX gives the quantity of all the elements present in Shankhapani Rasa. The quantity of elements analysed in EDAX are Hg-4.52%, Ca -12.79%, S-3.74%, Cl-14.11%, Na-8.84% and K-9.77%. The results show that the drug contains Mercury and Sulphur in very less quantity whereas Calcium and other organic elements are present in large quantities.

CONCLUSION

Analytical study on Shankhapani Rasa shows that it contains large amount of moisture 8.63% due to presence of organic matter. pH value 7.3., Total Ash value 66.63%, Water Soluble Ash value 41.60 %, Acid insoluble Ash value 17.22%. XRD reports show the presence of Meta Cinnabar in major phase and Sulphur in minor phase. The Scanning Electron Microscopy (SEM) shows the average crystallite size of drug as 77.97 nanometers at 7K which accounts for its bioavailability and efficacy. The quantity of elements analyzed in EDS is Hg-4.52%, Ca-12.79%, S-3.74%, Cl-14.11%, Na-8.84% and K-9.77%. These analytical values may be considered as standard parameters for maintaining the quality of Shankhapani Rasa.

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