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Pharamacaeutico- Analytical Evaluation of Kaseesa Bhasma

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ABSTRACT

Bhasmas are unique Ayurvedic preparations of metals/minerals formulated with herbal extracts or juices and used for the treatment of a variety of ailments. Owing to their micro/ nano fineness, ease of administration and comparatively small dose, they have been known for their vast area of application and therapeutic value. Kaseesa (FeSO4.7H2O) is an iron containing used in the treatment of eye diseases, pruritis, leucoderma, dysurea, helminthiasis. diabetes The present study was conducted to perform physicochemical characterization for the Kaseesa Bhasma as per the procedure mentioned in the literature by using several analytical tools. Kaseesa was taken for preparation of Bhasma with the help of Shodhana and Marana procedures as per traditional references. The Kasisa Bhasma were distinguished for their physicochemical characters using Energy Dispersive X-ray Spectroscopy (EDAX), X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM) analysis. EDAX analysis revealed that iron, sulfur, and oxygen was found to be71.62%, 0.52%, 27.81% respectively in Kaseesa Bhasma, The XRD pattern of Kaseesa Bhasma revealed that all the strong medium and weak peaks correspond only to Fe2O3. And its crystal structure is in rhombohedral in nature, the particle size of Kaseesa Bhasma using Z-average were found to be 798nm respectively.

KEYWORDS: *Bhasma, Kasisa, EDAX, SEM, XRD, NPST*

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INTRODUCTION

Rasashastra is the science dealing with Rasa dravya and its processing. This is a branch of Alchemy in which there is extensive use of metals and Minerals Since Vedic period metals have been used for welfare of human beings. In rasashastra classical literature has documented several texts which make sure the suitable transformation of essential metal into bio-absorbable calcined (Bhasma) form. Bhasma is the calcined preparation of minerals and metals having various advantages over herbal formulations2. Bhasmas are generally used in the healing of diseases because they have vast therapeutic value due to its easily assimilated, micro fineness, little dose and also great therapeutic effect. The preparation of Bhasma was a sophisticated process relating purification of the drug, levigation with different herbal juices, and formation of pellet along with incineration (Puta). But in the current scenario, certain people raised questions regarding the quality, efficacy and safety issues of Bhasma. It is recommended to use the advanced modern technology to make sure the suitable formation of Bhasmas. Kaseesa (green vitriol) is an iron containing Ayurvedic formulation, composed of ferrous sulphate (FeSO4. 7 H2O) used in the treatment of eye diseases, pruritis, leucoderma, dysurea, helminthiasis, fever, splenomegaly, primary and secondary amenorrhea, diabetes. The crude form of Kaseesa contains iron along with various other toxic metals and minerals which if taken without purification may produce various poorly effects within the body like palpitation, insomnia, burning sensation. So Kaseesa Bhasma was used as a medicine for treatment of various diseases after proper purification and incineration process. In addition to the main metal (Iron), several other metals are also expected during the preparation of Bhasma. This may be present in its elemental form during pharmaceutical processing. In addition, different pharmaceutical procedures used for the different manufacturing companies produce similar Bhasma with the diverse characteristic. This may lead to the difference in the metal content of samples which have

to contain these types of Bhasma. For this motive, it is necessary to standardize such type of Bhasma on the basis of their classical tests as well as using the advanced analytical parameters like, XRD, SEM, etc. The present study was conducted to perform physicochemical characterization for the Kaseesa Bhasma as per the procedure mentioned in the literature by using several analytical tools.

AIMS AND OBJECTIVE:

The present study has been carried out designed with following aims and objective.

1. To prepare kaseesa bhasma as per classical method
2. To analyse sample of Kaseesa Bhasma as per classical and modern parameter

MATERIAL AND METHODS:

The pharmaceutical study was conducted was conducted in department of Rasashastra and Bhaishajya Kalpana Taranath Government Ayurvedic Medical College and Hospital Karnataka Bellary. The Analytical parameter was conducted at Government college of pharmacy and Nannowatt technology Bengalore

Pharmaceutical study:

Procurement and authentication of raw materials:

Raw Kaseesa was procured from a genuine supplier Kajrekar Belgavi and authenticated as per their accepted characters (Grahya Lakshana) specified in the authoritative texts of Rasa-Shastra., Bhrinharaja and Lemon is collected from Bellary Locality

Preparation of Kaseesa Bhasma involve the following steps:

- Shodhana of Kaseesa
- Marana of Kaseesa
- **Shodhana of Kaseesa**[(1)]**:** shodhana of kaseesa was done as per Rasatantra sara va siddha prayoga Sangraha, At first Bhringaraja swarasa was prepared for kaseesa shodhana as per sharangadhara Samhita,

500gms of Kaseesa was taken in a clean dry Khalva Yantra and powdered. This powdered Kaseesa was added with 200 ml of Bhringaraja Swarasa (sufficient to soak Kaseesa), It was then triturated thoroughly till the liquid portion evaporates., Later it was kept in sunlight for 2 hours to dry the drug completely. Again the same process was repeated for one more times for 2nd day Bhavana with 150ml of Bhringaraja Swarasa each. Every time freshly prepared Bhringaraja Swarasa was used

Kaseesa was light green colour before Bhavana. It was changed to dark green immediately when Bhringaraja Swarasa was added. Odour of Bhringaraja Swarasa was appreciated throughout the process of Bhavana. After Bhavana Kaseesa changed from dark green to light green colour. When it was dried in sunlight it attained dull white colour.

- **Marana of Kaseesa**[(2)]**:**

Marana of kaseesa was done as per Rasa ratna Samucchaya

The Kasisa Bhasma was prepared by incineration process in this process 520gm of Shodhita Kasisa was kept in a mortar and triturate with required quantity of Nimbu swarasa. After trituration uniform size, thick and small pellets of Kasisa was prepared. The sun dried pellets of Kasisa were kept in an earthen pot. The earthen pots were smeared seven times with mud and cloth and subjected to the heating system (Laghuputa). This process was repeated for seven times after that bright red colour of Kaisa Bhasma was formed.

Physicochemical evaluation: Raw, Shodhita Kasisa and Bhasma were analyzed by using organoleptic parameters, Bhasma Pariksha (Test of accurately prepared Bhasma), Scanning electron microscope, Energy dispersive X-ray analysis, and X-ray diffraction analysis.

Bhasma Pariksha: Bhasma Pariksha is a very important part in the field of Ayurveda to ascertain the fact that the prepared Bhasma are best in quality so that maximum therapeutic results can be obtained from it. Therefore, to conclude the fact that Bhasma prepared are standard and genuine, in the present study the kaseesa bhasma is passed all prescribed tests. a) Rekhapuranatva (enterabilty in the furrows of the finger tips) b) Varitaratva (Floatabilty on still surface of water) c)) Mridutva (softness on touching) d) Slakshanatva (smoothness to touch) e) Gatarasatwa (Tastelessness) f) VisistaVarnotpotti (development of specific colour) and also Raw, Shodita and Kaseesa Bhasma underwent a analysis of Modern parameters i, e physical property (organoleptic characters) likeTaste, Colour, Touch, Taste, Consistency., P^{H}, Determination of loss on drying AT 1050C:) Determination of ash value, Determination of acid insoluble ash, Determination of water soluble ashTotal microbial count

INSTRUMENTAL ANALYSIS[(3)]**:**

1. **X-ray Diffraction**
2. **Scanning Electron Microscopy (EDX) Study:**
3. **Particle size analysis**:
4. **Namboori phased spot test (npst):**

Results:

Table No 1.: Showing the Results *Kaseesa* Shodhana:

No of bhavana	Quantity of swaras used	Weight of *Kaseesa* before bhavana	Weight of *Kaseesa* after bhavana	Duraion of bhavana
1st bhavana	350 ml	500gm	539gm	12hrs

Total yield of Shodita *Kaseesa* is 39gm.

Table No 2: Showing the Results *Kaseesa* Maran

At puta	Wt. of *Kaseesa* before Bhavana (gms)	Quantity of Nimbu swarasa required	Time required to get chakrika Consistency	Wt. of *Kaseesa* after puta (gm)	Loss inwt. (gm)	Total loss inwt.	Total loss in wt. in %
1	520gm	900ml	4hr, 30min.	420gm	100gm	422. 5gm	81. 25%
2	420gm	800ml	4hr, 15min.	380gm	40gm		
3	380gm	750ml	4hr	355. 5gm	24. 5gm		
4	355. 5gm	640ml	4hr	315gm	40. 5gm		
5	315gm	620ml	4hr	295gm	20gm		
6	296gm	580ml	4hr	235gm	60gm		
7	235gm	520ml	4hr	179gm	56gm		
8	179gm	460ml	4hr	147. 5gm	31. 5gm		
9	147. 5gm	420ml	4hr	97. 5gm	50gm		

Table No. 3: Changes observed in pellets after each *Puta*

At *Puta*	Colour	Odour	Touch	Taste	Consistency	*Varitara*	*Rekhapurana*
1	Brownish black	No typical odour was present	Rough	Metallic and sour taste	Hard	-ve	-ve
2	Brownish black	No typical odour was present	Rough	Metallic &sour taste	Hard	-ve	-ve
3	Brownish black	No typical odour was present	Rough	Metallic &sour taste	Hard	-ve	-ve
4	Slightly red	No typical odour was present	Rough	Metallic &sour taste	Hard	-ve	-ve
5	Slightly red	No typical odour was present	Soft	Metallic taste reduced & sour taste Persists	Hardness slightly reduced	-ve	+ve
6	Ishtikavarna	No typical odour was present	Soft	Metallic taster educed & sour taste Persists	Hardness reduced	-ve	+ve
7	Ishtikavarna	No typical odour was present	Soft	Metallic taste reduced & sour taste Persists	Soft	-ve	+ve
8	Gairikavarna	No typical odour was present	Soft	Metallic taste reduced & sour taste Persists	Soft	-ve	+ve
9	Gairikavarna	No typical odour was present	Soft	No specific Taste	Soft	+ve	+ve

Table No. 4: Showing average Time and Temperature

Sl. No.	Time (hr: min)	Temperature in ^{0}C.
1	00:00	29^{0}C
2	00:15	56 ^{0}C
3	00:30	100^{0}C
4	00:45	156^{0}C
5	01:00	182^{0}C

6	01:15	220^{0}C
7	01:30	236^{0}C
8	01:45	260^{0}C
9	02:00	292^{0}C
10	02:15	312^{0}C
11	02:30	334^{0}C
12	02:45	364^{0}C
13	03:00	396^{0}C
14	03:15	440^{0}C
15	03:30	458^{0}C
16	03:45	480^{0}C
17	04:00	478^{0}C
18	04:15	474^{0}C
19	04:30	470^{0}C
20	04:45	464^{0}C
21	05:00	430^{0}C
22	05:15	382^{0}C
23	05:30	364^{0}C
24	05:45	332^{0}C
25	06:00	298^{0}C
26	07:00	250^{0}C
27	08:00	238^{0}C
28	09:00	182^{0}C
29	10:00	156^{0}C
30	11:00	112^{0}C
31	12:00	108^{0}C
32	24:00	31^{0}C

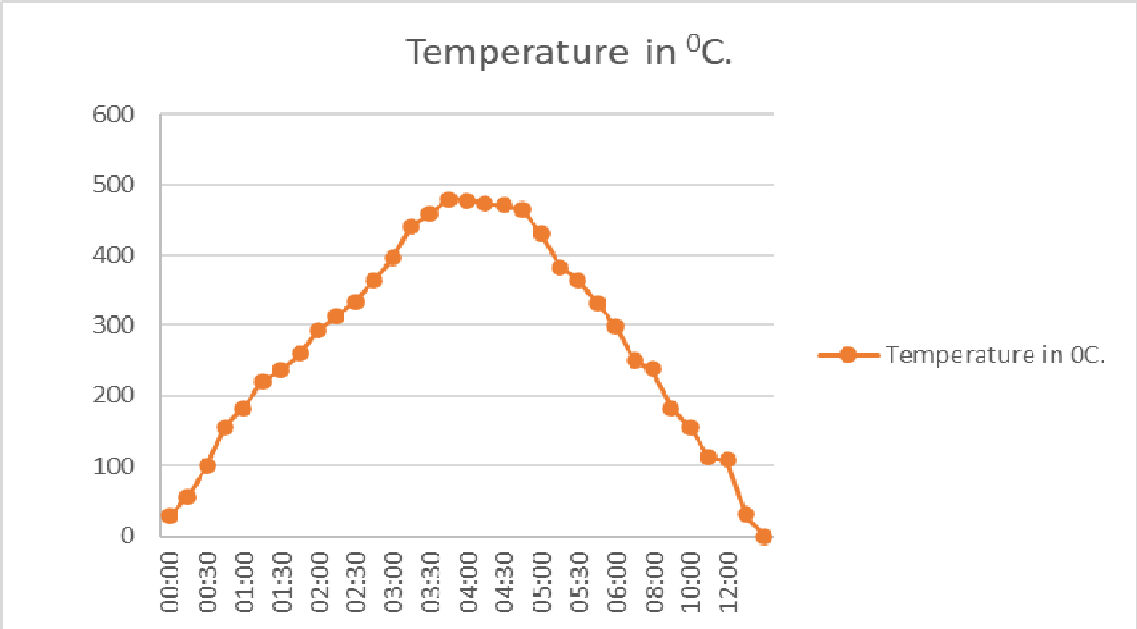


Figure 1: TGA analysis of Kasisa Bhasma

Table No 5.: Showing Classical Parameters for *Kaseesa Bhasma*:

Sl. No	Parameters	*KaseesaBhasma*
1	*Rasa*	*Niramla*
2	*Varna*	Reddish brown
3	*Sparsha*	Smooth, fine (*Mrudu)*
4	*Gandha*	*Nirgandha*
5	*Varitaratva*	Positive
6	*Rekhapoornatva*	Positive

Table No 6: Showing the result of Organoleptic Characters of *Kaseesa Bhasma*:

Sl. No	Parameters	*Kaseesa Bhasma*
1.	Colour	Reddish brown
2.	Odour	Non Specific
3.	Touch	Soft
4.	Taste	Sourless
5.	Consistency	Soft, microfine

TABLE NO 7: Showing the result of physical test of *RK, SK, KB*:

PARAMETERS	*RK*	*SK*	*KB*
Ph value	2.74	2.82	5.6
LOD at 105^0C	31.37%,	34.42%,	3.05%
Total ash value	62.34%,	57.19%,	34.5%
Wate soluble ash	28.76%,	26.63%,	26.63%,
Acid insoluble ash	21.68%,	14.18%,	11%

Table No 8: Showing the result of total microbial count of *RK*, *SK*, *KB*:

SLNO	Dilution	Number of colonies (NOC)			CFUg
1	$1/10(10^{-1})$	0	0	0	0
2	$1/100(10^{-2})$	0	0	0	0
3	$1/1000(10^{-3})$	0	0	0	0

Table No 9: Showing the result of total fungal count of *RK*, *SK*, *KB*:

SLNO	Dilution	Number of colonies (NOC)			CFUg
1	$1/10(10^{-1})$	0	0	0	0
2	$1/100(10^{-2})$	0	0	0	0
3	$1/1000(10^{-3})$	0	0	0	0

Table No 10: showing XRD report of *KB* compared with standard of hematite(Fe_2O_3):

Identified				Standard		
Peak no	Angle 2 θ	d space	Intensity	Angle 2 θ	d space	Intensity
01	24.372	3.6559	4181	24.16	3.680	3192
03	33.602	2.6649	6419	33.280	2.690	6817
04	35.921	2.4723	6593	35.744	2.510	5257
07	41.332	2.1826	4446	40.991	2.200	4126
09	49.853	1.8283	4872	49.498	1.840	8234
11	54.473	1.6831	5081	54.233	1.690	9093
14	62.589	1.4829	4842	62.260	1.490	8926
17	64.522	1.4431	4788	64.129	1.450	8912
Name of standard: Haematite Crystal structure: Rhombohedral JCPDS No: 00-001-1053 Mwt: 159.69g/mol.						

Table No 11: - Showing SEM-EDX of *Kaseesa Bhasma*

El Element	M Mass%	At Atomic%
O	72 27. 81	29 57, 21
S	27 0. 52	69 0. 54
Fe	0. 71. 67	0. 42. 24
Total	1 100. 00	

Table No 12: - Showing Particle Size of *KB*:

Effective diameter		
Sample	Z-Average (d. nm)	Pdl
KB	798. 9	O. 376

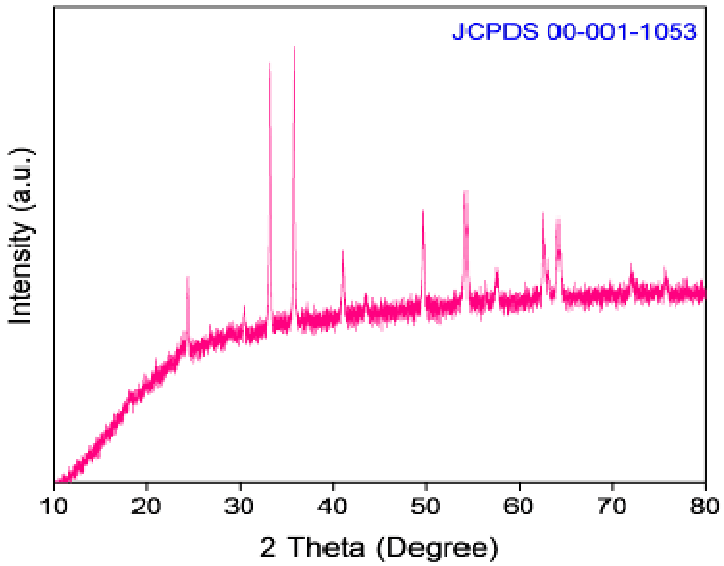


Figure 1: XRD-Analysis of Kaseesa Bhasma

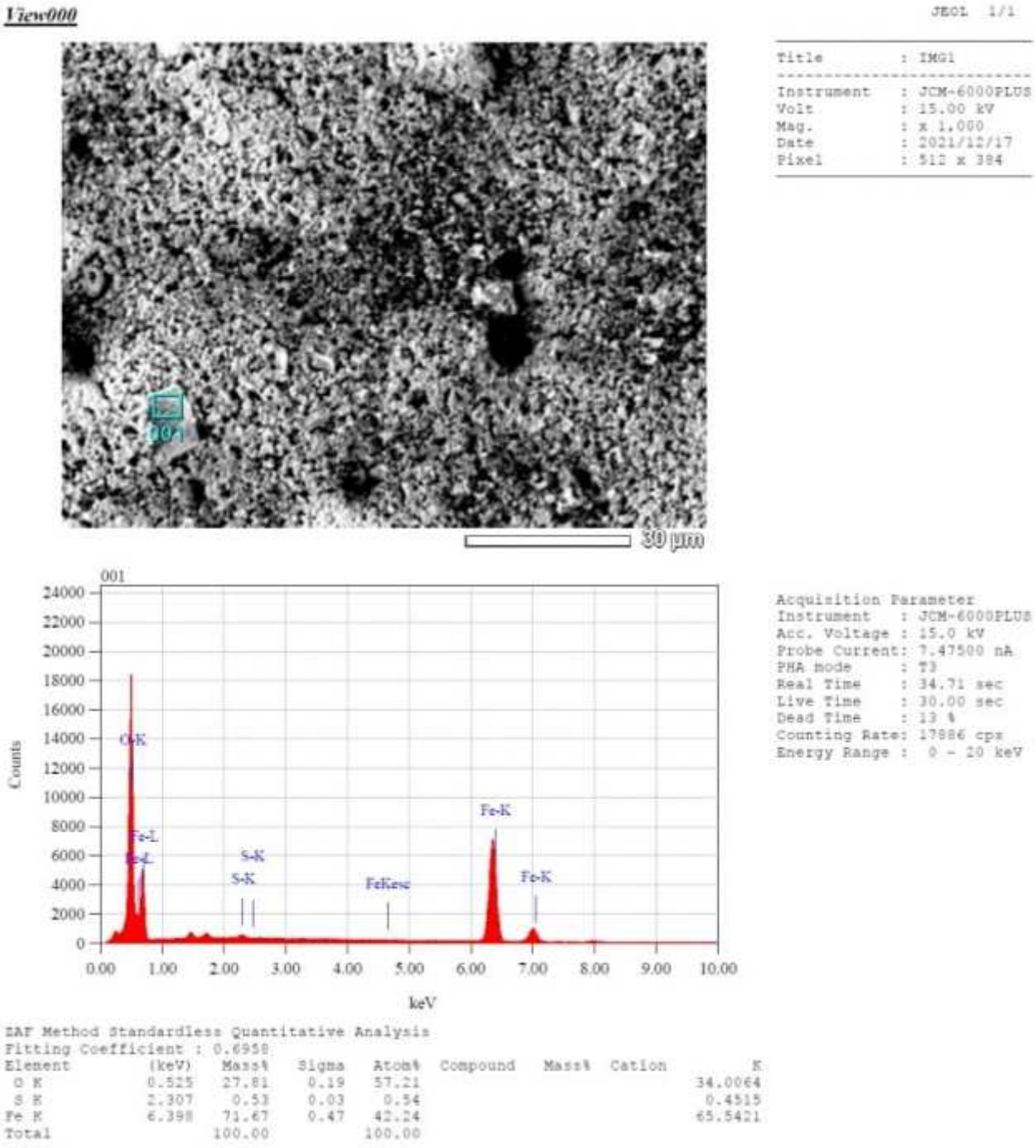
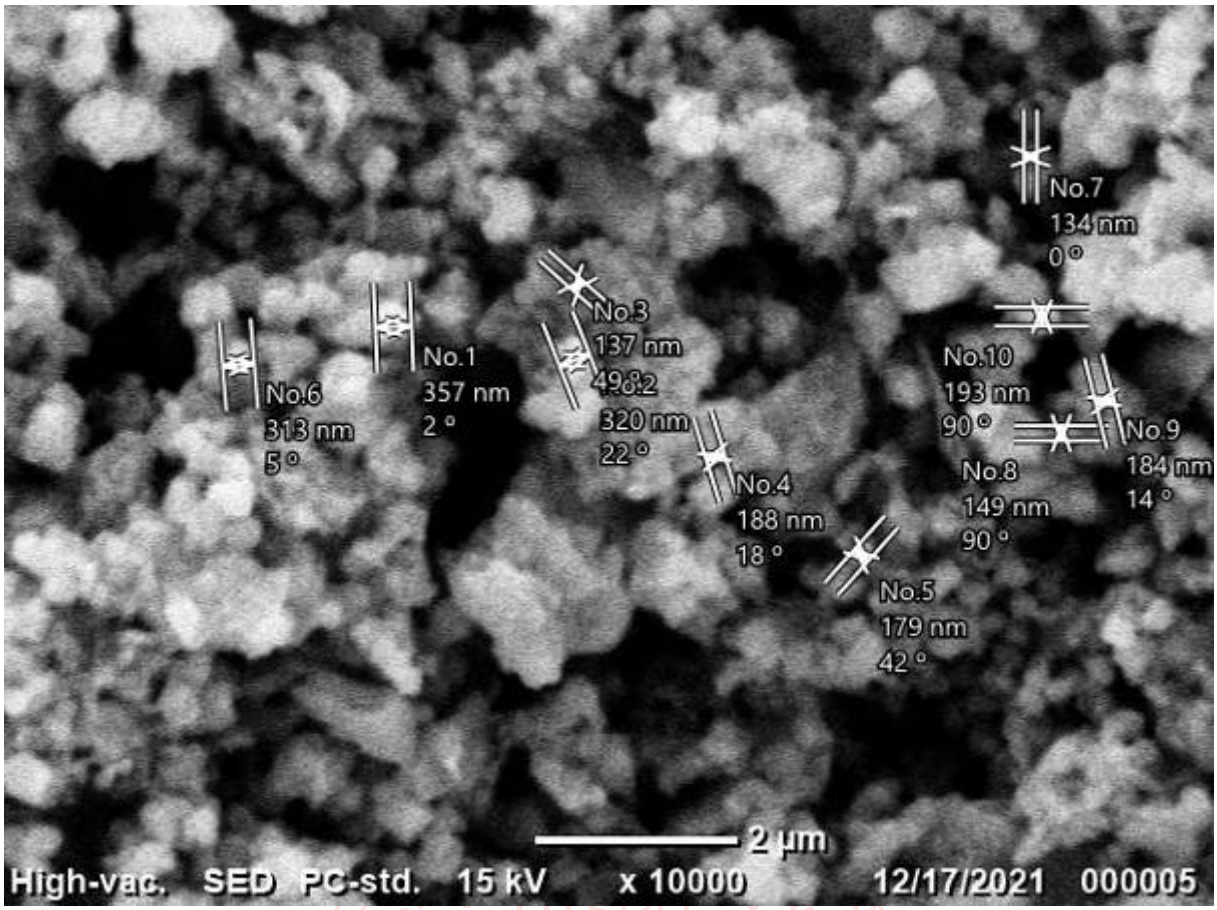
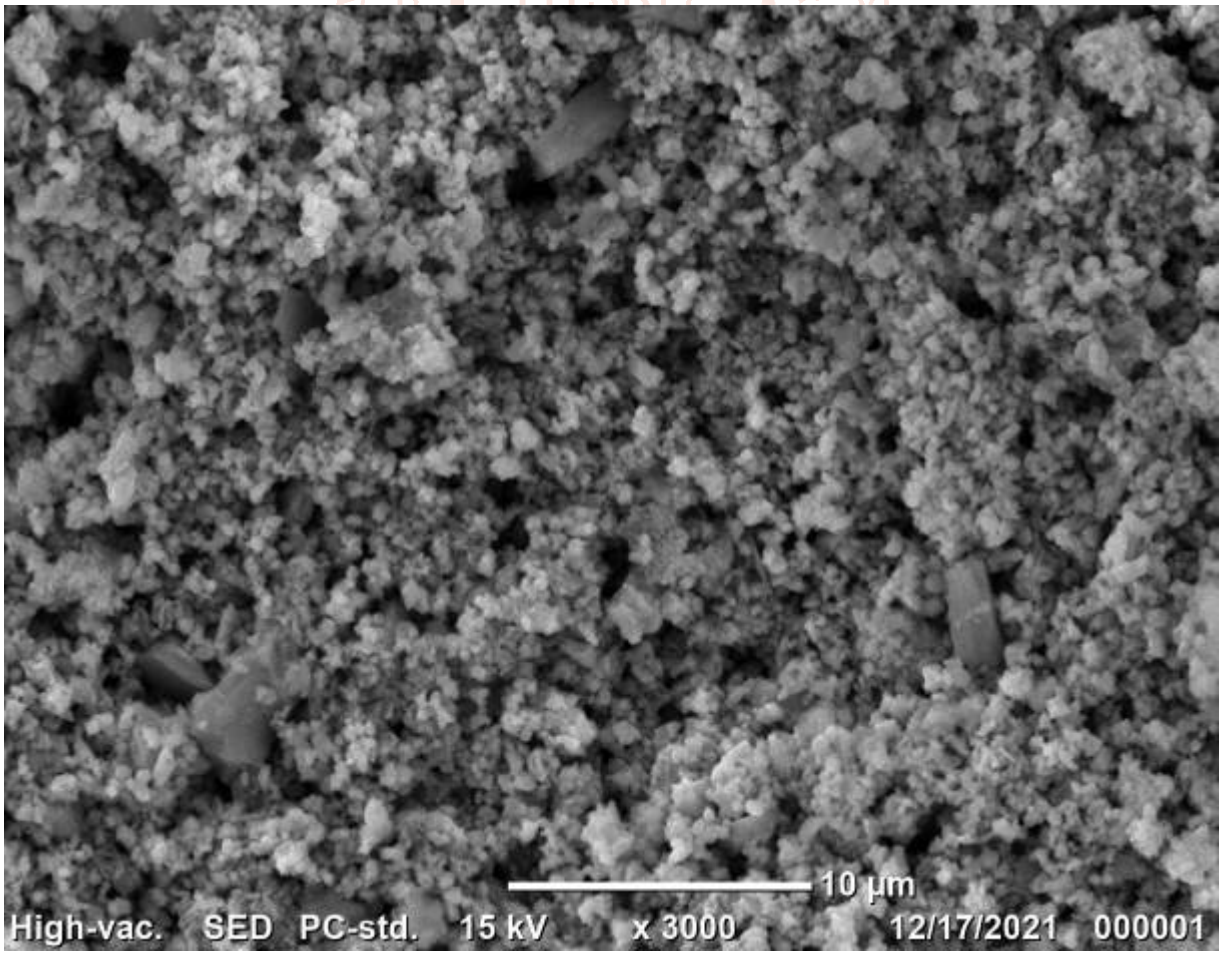


Figure 2: SEM-Analysis of Kaseesa Bhasma


No.7
134 nm
0 °
No.3
137 nm
49.2
320 nm
22 °
No.1
357 nm
2 °
No.6
313 nm
5 °
No.10
193 nm
90 °
No.9
184 nm
14 °
No.8
149 nm
90 °
No.4
188 nm
18 °
No.5
179 nm
42 °
2 µm
High-vac. SED PC-std. 15 kV x 10000 12/17/2021 000005


10 µm
High-vac. SED PC-std. 15 kV x 3000 12/17/2021 000001

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: Kaseesa PSA
SOP Name: mansettings.nano
General Notes:

File Name:	Y1.dts	Dispersant Name:	Water
Record Number:	1	Dispersant RI:	1.330
Material RI:	1.46	Viscosity (cP):	0.8872
Material Absorbtion:	0.010	Measurement Date and Time:	05 February 2022 11:57:19

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	354.0	Measurement Position (mm):	4.20
Cell Description:	Low volume glass cuvette (4...	Attenuator:	10

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	798.9	Peak 1:	786.5	100.0	142.4
PdI:	0.376	Peak 2:	0.000	0.0	0.000
Intercept:	0.855	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

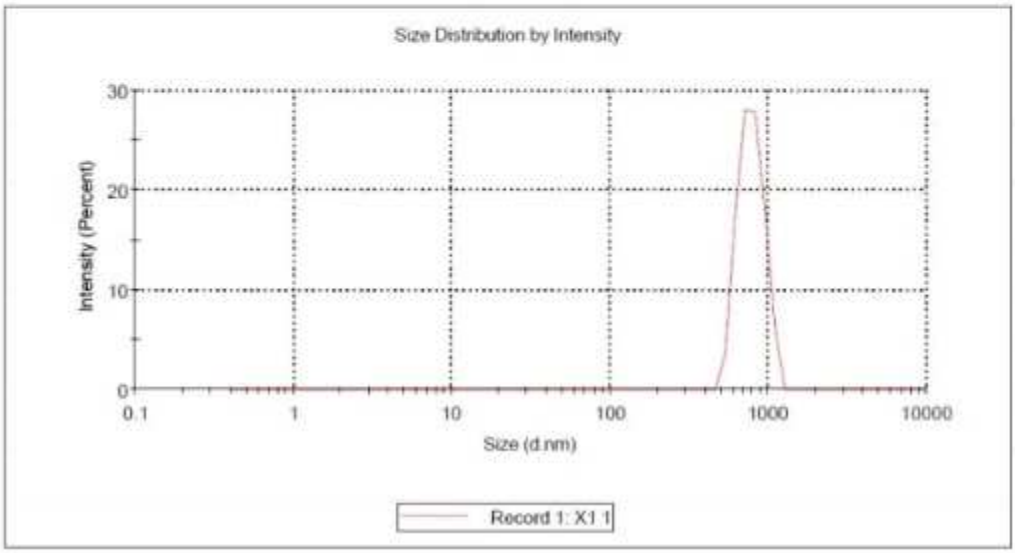


Figure 3: Particle size-Analysis of Shodhita Kasisa

IMAGES:

Fig 1 Raw Kaseesa

Fig 4-ingradients for kaseesa sshodhana

Fig: 2 Bhringaraja

Fig: 5 Subhavita lakshana of Kaseesa

Fig: 3 Bhringaraja Swarasa

Fig: 6 Nimbuka

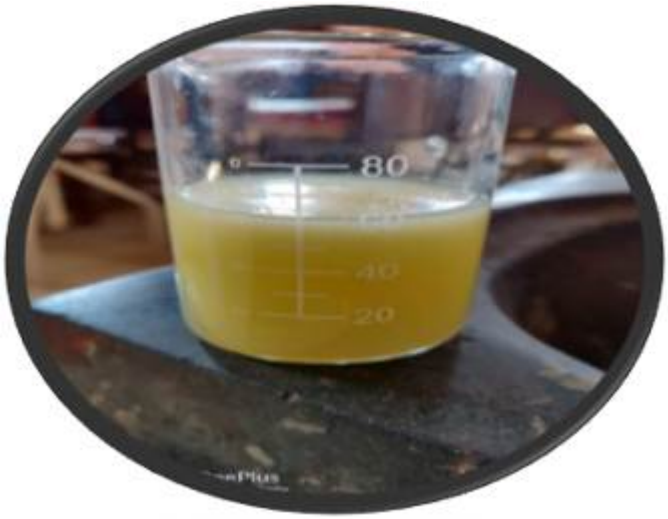


Fig: 7 Nimbu Swarasa

Fig: 8 Bhavana of Shodita Kaseesa

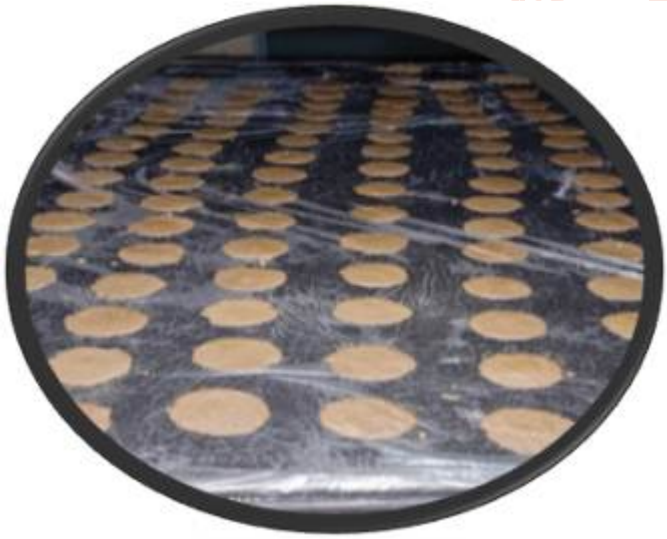


Fig: 9 Chakrikas of Kaseesa

Fig: 10 Colour of Chakrikas on 6th puta

Fig: 11 Colour of Chakrikas on 7th puta

Fig: 12 Varitaratwa of Kaseesa Bhasma

Fig: 13 Rekhapurnata of KaseesaBhasma

Fig: 14 Materials required for Bhasma Pareeksha

Fig: 15 NPST 1st phase of KB

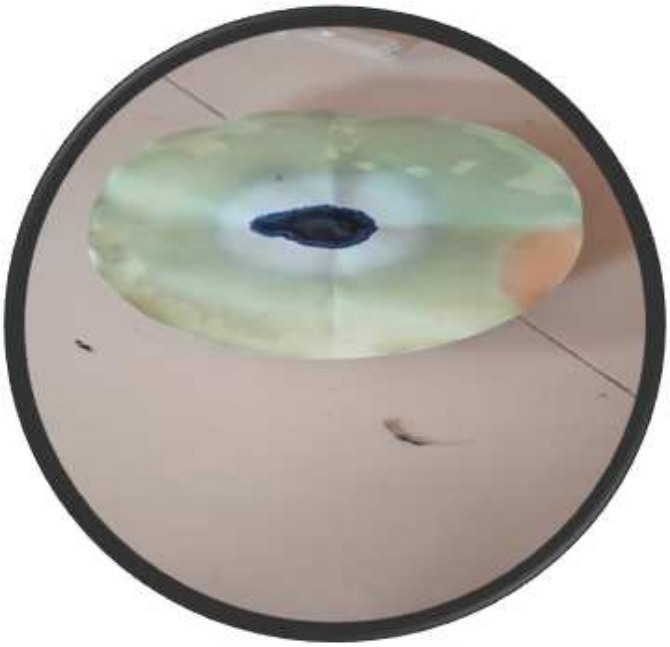


Fig: 16 NPST 2nd phase of KB

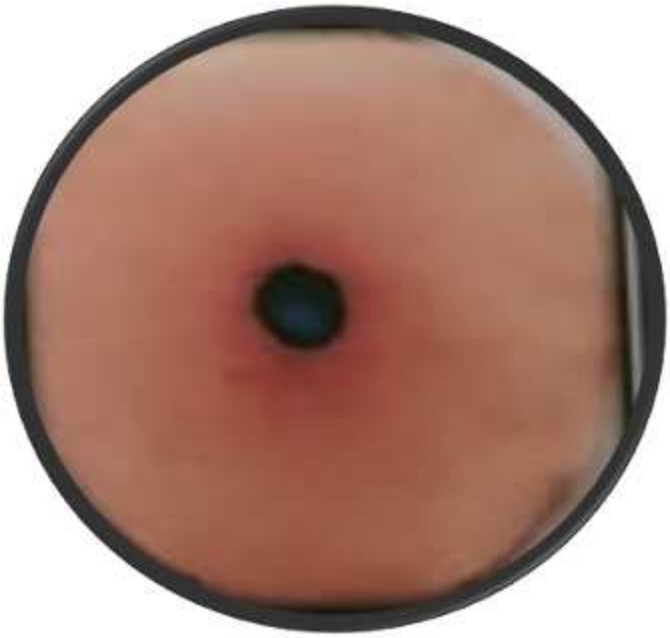


Fig: 17 NPST 3rd phase of KB

Discussion:

Efforts were made to prepare Kasisa Bhasma from raw Kasisa by adopting a classical reference of 'Rasa Tantra sara va siddha prayoga sangraha and Rasa Ratna samucchaya 'for its Shodhana and Marana purpose. The first step of pharmaceutical processing of Kasisa Bhasma is Shodhana of raw material. The objective of Kasisa Sodhana is mainly to remove unwanted materials from Kasisa and to enhance its potency. In the present study, Bhavana method was adopted in Shodhana process

Discussion on Shodhana of Kaseesa: in the present study, it was done in one batch weighing 500 gm for practical convenience. Two Bhavana of Bhringaraja Swarasawere given to Kaseesa. for 12hrs, In this process an average of 39 gm gain was found during

Bhavana. After second Bhavana of Bhringaraja Swarasa Kaseesa obtained was 539gm. Bhavana of total 350 ml of Bhringaraja Swarasa was given to Kaseesa. After Bhavana, when it was in wet form an average of 78gm weight gain was observed i, e 578gms due to the addition of Bhringaraja Swarasa but later when it was subjected for drying in sunlight due to evaporation of water content from the Kaseesa, (Table no 1) around 39 gm of weight loss was observed and net gain of shodhita Kaseesa is 539gms, around 19gms of shodhita Kaseesa was taken for physico-chemical analysis.

Discussion on Marana of Kaseesa: For the present study, it was done in one batches weighing 520 gm, Shuddha Kaseesa was triturated with sufficient quantity of Nimbu Swarasa and trituration was done for four and half hours till it became thick paste to prepare Chakrika. Chakrikas were having 4 cm in diameter and 0. 5 cm thickness because every particle should get adequate heat for incineration. Chakrika should be placed directly in Sharava to avoid loss. For drying of Chakrika, it took long time. It may be because Kaseesai. e. ferrous sulphate contains water of crystallization and it has tendency to absorb moisture from air. Acharya Yadavaji Trikamji in his book Rasamrita especially, remarked that Chakrika should be dried well, because wet Chakrika after subjecting to Puta causes blackening of Bhasma. In Kaseesa Bhasma Vishista Varna (Gairikavarna) and Niramlatwa is the main test. After drying of Chakrika, Sharava Samputikarana was done and subjected to laghu Puta around 16 Vanyopala taken for Puta. After Puta kept it for swanga sheeta, after swanga sheeta maarita Kaseesa was collected and subjected it into next Bhavana with Nimbu Swarasa was given. Chakrika were prepared after trituration, subjected to Puta. Total 9 laghu Puta were given. After 1st, 2nd, 3rd Puta, It was observed that Kaseesa Chakrika was Brownish black in colour and rough to touch, and amlatwa taste was appreciated, varitara was not observed, on 4th Puta Kaseesa Chakrika was slightly red in colour, rough to touch, and amlatwa taste was appreciated, varitara was not observed, on 5th puta Kaseesa Chakrika slightly red in colour and soft in touch, varitara was partially observed, on 6th and 7th puta Kaseesa Chakrika becomes ishtikavarna, and soft to touch, hardness of chakrikas were reduced, on 8th puta Kaseesa Chakrika becomes gairikavarna, 90% varitara was observed, on 9th puta Kaseesa Chakrika attains gairikavarna, complete Rekhapurnata, varitaratwa was observed, no specific taste was observed (Niramlatva rasa of Kaseesa was appreciated). Total 97. 5gm Kaseesa Bhasma was obtained from 520gm of Shuddha Kaseesa(Table no 2). That means average 81. 25% of loss was observed. Due to the presence of water molecules, the oxidation process of Kaseesa is more compare to other uparasa rasa dravyas, hence the yield is less in percent.

Discussion on Elemental Analysis:

Discussion on XRD: Total 25 peaks were identified from angle (2θ) 24. 372 to 64. 522 9 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file. 1 nd peak with relative intensity of 4181 was significant at angle 24. 372 having 3. 6559 d space values. 2θ values of standard Haematite (24. 16, 33. 280, 35. 744, 40. 991, 49. 498, 54. 233, 62. 260, 64. 129) are almost similar to identified Kaseesa Bhasma values (24. 372, 33. 602, 35. 921, 41. 332, 49. 853, 54. 473, 62. 589, 64. 522) respectively. D-space values of standard Haematite (3. 680, 2. 690, 2. 510, 2. 200, 1. 840, 1. 690, 1. 490, 1. 450) are almost similar to identified KaseesaBhasma values (3. 6559, 2. 6649, 2. 4723, 2. 1826, 1. 8283, 1. 6831, 1. 4829, 1. 4431) respectively (Table no 10). These peaks of KB sample which were compared with standard Angle 2 θ JCPDF values confirmed that the presence of Haematite and: Rhombohedral Crystal structure.

Discussion on SEM-EDX: EDX is the popular technique used for the estimation of macro-elements in the herbo-mineral formulation. In present work this instrument technique was especially applied for the estimation of elements present in KB is Ferrous & Sulphur The percentage of Ferrous has been increased in KB i. e 71. 67 compared to Sulphur percentage which is 0. 52. The percentage of sulphur decreased in KB compared to Ferrous(Table no 11), it may be due to the carbonization of organic substances during Marana karma. The above change in the percentage of elements may be due to the heat treatment, which causes breaking of bond and the formation of new bond with the evaporation of certain gases like SO2, CO2 resulting in the increase and decrease of other elements.

Discussion on Particle size: Particle size helps to know the absorption of drug. Lesser the particle size more is the surface area of absorption. Nanoparticles gained importance in medical field because of their surface to mass ratio much larger than that of other particles. Particle size reduction will result in precise drug delivery and thereby increasing the bioavailability of the drug. Mean particle sixe of KB is798. 9nm. (Table no 12) The reduction in particle size of compared to may be due to Bhavana and marana karma.

Discussion on NPST: N. P. S. T is a modification of circular paper chromatography. N. P. S. T gives a clear differentiation of individual products in a group

and also product can be identified by its classical name not by the chemical name. The continual chemical reactions taking place gradually between 2 chemical substances on static media at fraction of second and also after certain interval of time are easily detected by their distinct colour changes and the pattern of spot. NPST was carried out with an intention to find the chromatographic standards of Kaseesa Bhasma. It was observed that the pattern of bluish colour changes exactly tallies the standard pattern of Kaseesa Bhasma indicating high standards. The series of changes in the colour is due to reaction between Potassium ferrocyanide paper and solution prepared out of KB.

CONCLUSION Conclusions are drawn based on the observation and interpretations made during the whole study.

Kaseesa Bhasma is one of the Bhasma kalpana here Rasa Tantra Sara va Siddha Prayoga Sangraha reference was selected for the preparation of Kaseesa Bhasma. Kaseesa Shodhana was done with Bhringaraja swarasa for 12hrs and marana by Nimbu swarasa and subjected to 3 laghuputas, and Jambu Beeja Choorna was prepared according to Sharangadhara reference. Kaseesa Bhasma prepared was 97. 5gm

XRD validates the chemical nature of KB i.e (Fe_2O_3) as major compound in crystalline phase was present in KB final product.

Particle size Analysis reveals presence of nanoparticles in KB final product is 798. 9 nm which will be concluded best absorbed & increases the bioavailability & easily assimilable form.

Elements present in KB are Oxygen-27. 81%,, Sulphur-0. 52%, Ferrous71. 67% as confirmed by the EDX Study.

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