IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 6, Issue 4 (May. – Jun. 2013), PP 05-12
www.iosrjournals.org

Chemical Investigations of Some Commercial Samples of Calcium Based Ayurvedic Drug of Marine Origin: *Kapardika Bhasma*

Sonali Dhamal, M.P.Wadekar*, B.A.Kulkarni, V.V.Dhapte
Department of Chemistry, Yashwantrao Mohite College, Bharati Vidyapeeth Deemed University, Pune-411038, Maharashtra, India.

Abstract: *Kapardika bhasma is an important Ayurvedic drug of marine origin. Even though it is composed of mainly of calcium carbonate it exhibits excellent medicinal properties which are not associated with standard calcium carbonate. In the present study four commercial samples are characterized using techniques like EDX, SEM, IR, UV,XRD and TG analysis to throw light on their chemical composition and chemical properties .Such comparative study may help to standardise and to interpret the biological and medicinal properties of such traditional drug.*
Keywords: *Calcium carbonate, Kapardika bhasma, TG, SEM, IR, XRD.*

I Introduction

Ocean is the biggest natural repository providing a huge number of materials many of which are pharmaceutically important. Among these, there is a specific group of marine products of animal origin whose pharmaceutical utility and medicinal importance was recognized long ago by traditional pharmacists. This group includes five calcium based materials namely Counch [*terbinella rapa*], Counch shell *[xan thus pyrum*], *Kapardika* [*cypraea moneta*], Praval [*coralliun rubrum*] and Pearl *[pinctada margarifiera*].
In Indian systems of medicine a huge number of pharmaceutical preparations are established according to procedures reported in ancient ayurvedic texts. *Kapardika bhasma* is one of these drugs which is the subject of present investigation.

Kapardika Bhasma is a valuable Ayurvedic drug which is used as powerful antacid and many stomach problems. It is prepare from *Kapardika* powder. Various methods are reported in Ancient Ayurvedic literature such as Rasatarangani, Charak Sanhita. These procedures are followed by Ayurvedic pharmacy still today.
Therefore, most of these formulations are considered as traditional medicines and they are not acceptable as approved drugs on international level. Actually due to their natural and biological origin they possess several merits as compared to their equivalent drugs in current use. Economically also, they are much cheaper due to easily available raw material in huge quantity at a lower cost. Hence if their pharmaceutical and biomedical chemistry is reinvestigated by standardizing synthetic procedures and methods of characterization on the basis of modern analytical techniques, all these formulations will receive international appreciation and acceptance on wider scale.

Literature survey reveals that studies on chemical investigations of some Ayurvedic *bhasmas* of mineral origin are reported [1-4] but relatively less work is reported on *bhasmas* of marine origin. A review on *Kapardika bhasma* is reported by Krishna *etal* [5]. Some structural characterization on *Varatika bhasma* (i.e. *Kapardika bhasma*) is also reported by Devanathan *etal* [6]. Some calcium based Ayurvedic *bhasmas* are analysed by instrumental neutron activation analysis for major and minor constituent elements by Kumar *etal* [7]. An attempt has been made by them to correlate metallic contents with their medicinal importance. Antacid activity of some Ayurvedic calcium preparations is studied by Baxi [8].
In the present study Chemical investigations of some commercial samples of *Kapardika bhasma* are done using modern techniques such as EDX, SEM, Solid state UV, Solid state IR, TGA and XRD. Such study may throw light on chemical composition as well as structural properties of *Kapardika bhasma* and hence the origin of its medicinal properties. Four samples from reputed pharmacies (K-1, K-2, K-3 and K-4) from different parts of India are selected for current study in order to understand the current status of these *bhasmas.*

II Materials and Methods

Kapardika bhasma (K1, K2, K3, K4) from four reputed pharmacies are collected for the present study. Standard $CaCO_3$ was purchased from Alderich for comparative purpose. Powder of *Kapardika* (K) was also purchased from Ayurvedic pharmacy.

Instrumental Techniques

Calcium carbonate, *Kapardika* powder and samples of *Kapardika bhasma* were analysed using EDX machine (JEOL JSM – 6360A Analytical SEM) for obtaining the relative percentage of constituent elements. The solid state infrared spectra were recorded in KBr (discs) in the region 4000-450 cm-1 on Perkin Elmer model 1600 and solid state UV-Visible spectra were recorded in the region (220 – 800 nm) against pure KBr disc as the reference. The KBr disc was prepared by mixing 1 mg of each sample with 100mg of KBr. Thickness of the disc was maintained 0.1 mm for each of the sample. The XRD patterns were recorded on a Rigaku (Geigerflex RB RU 200) X-ray diffractometer using Cu K alpha radiation filtered by a nickel foil over the range of diffraction angle 3-50^0, the wavelength used being 1.542 A^o. Thermograms of the sample are recorded on Perkin Elmer TG analyser up to 1000^{0}C at a heating rate 10^{0}C per minute in air atmosphere. SEM photographs of the samples were recorded on machine JEOL JSM – 6360A Analytical SEM.

III Results and Discussion

3.1 EDX-Analysis

The EDX analysis is mainly used to identify the constituent elements and for knowing the relative percentages of these elements. These results are expressed in terms of bar diagram shown in the Fig-1.

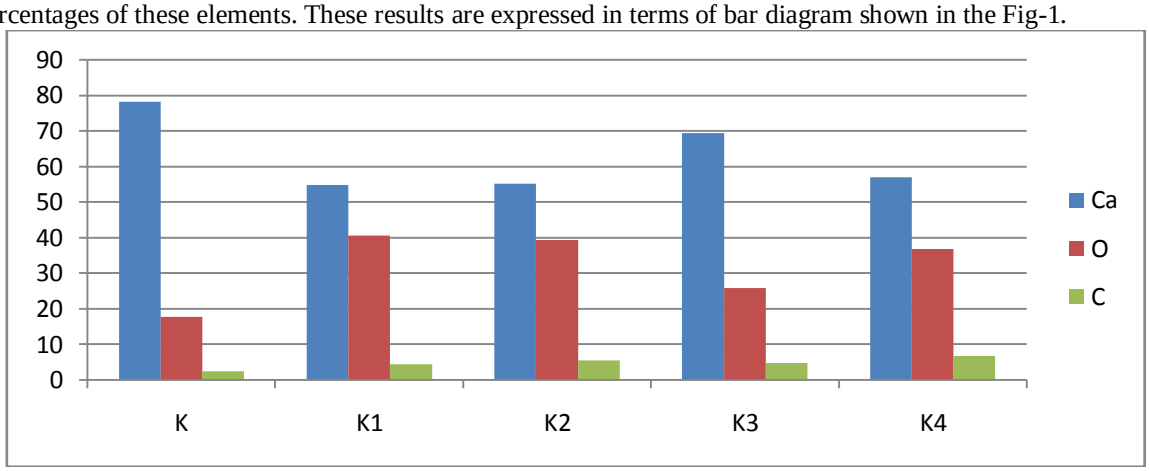


Fig-1 Bar Diagram showing relative percentage of constituent elements of *Kapardika bhasmas*

Pure and stoichiomertically correct $CaCO_3$ is composed of ~40% Ca and ~60%CO_3 but the commercial sample of $CaCO_3$ of AR grade under study is associated with MgO (0.4%) as the impurity. Naturally occurring *Kapardika* powder also contains predominantly calcium, carbon and oxygen. But it is associated with sodium as a minor constituent. The four samples of *Kapardika bhasma* are also more predominantly composed of the mixture of calcium, carbon and oxygen. This important observation is further supported by IR studies.

3.2 Infrared Spectroscopy

Infrared spectra of *Kapardika* powder and samples of *Kapardika bhasma* are compared with standard $CaCO_3$ because all these samples are mainly composed of $CaCO_3$ as observed from EDX. IR spectra of all the samples are presented in Fig-2 and important frequencies are summarized in Table-2.

Conclusions from IR Study:

1. The IR spectrum of *Kapardika* powder shows characteristic peaks in the region 600-750 cm^{-1} confirming presence of calcium carbonate [9]. Most of the samples of *Kapardika bhasma* also show two peaks in the same region.
2. In Synthetic $CaCO_3$ and in K-1, K-2, K-4 broad bands in region around 3445-3642 cm^{-1} are observed. They are either due to O-H from H_2O present in the lattice [10] or from $Ca(OH)_2$ associated with $CaCO_3$[11] of respective samples except that of K-3 sample.

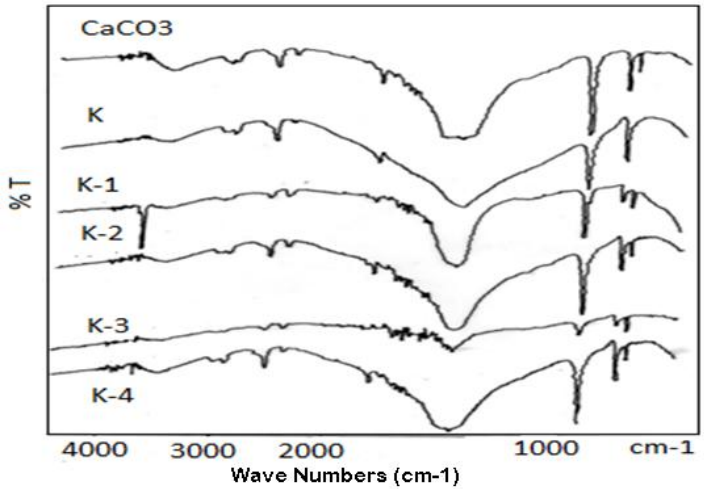


Fig -2 Infrared spectra of *Kapardika* powder and *bhasmas*.

Table-2 Infrared Frequencies of *Kapardika* Powder and *Kapardika Bhasmas*

Sr. No	Compound	Peak-1	Peak-2	Peak-3	Peak4	Peak-5	Peak-6	Peak-7	Peak-8	Peak-9
	Assignment	*O-H (H_2O)/ $Ca(OH)_2$*	*Organic mater*	*Organic mater*		*H-O-H*	*CO_3^{2-}*	*CO_3^{2-}*	*CO_3^{2-}*	
1.	$CaCO_3$	3445	2887 2872	2512	2360	1795	1403 1422	872	712	668
2	K	3446	2872	2512	2370	1799	1418	875	740 750	----
3	K-1	3642	----	2512	2360	1809	1435	910	712 750	----
4	K-2	3447	2875	2512	2344	1799	1436	875	712 750	600 620
5	K-3	----	----	2513	2330	1843	1436	874	712	668
6	K-4	3444 3673	2898	2509	2362	1791	1446	874	726 750	----

3. In case of the *Kapardika* powder the broad peak around 3446 cm^{-1} is assignable to lattice water and associated organic matter [12] which is further supported by thermal studies. In K-3 sample there is no indication of water molecule in its structure. In the spectrum of K-1 the peak around 3642cm^{-1} is sharp which is rather surprising. The bands around 1795-1809 cm^{-1} could be assignable to H-O-H of water molecule from $CaCO_3$ [9].
4. The broad peaks around 1422 cm^{-1} as well as sharp peaks around 872 and 772 cm^{-1} are due to carbonate ions (CO_3^{-2}) from $CaCO_3$ [9] in all five samples. The possible reasons for the presence of such peaks could be as follows. The preparation of *Kapardika bhasma* involves repeated treatment of *Kapardika* powder with medicinally important plants like Aloe Vera and repeated calcinations cycles in traditional furnace in an earthen pots where temperature in the furnace is between 600-900^{0}C [13,14]. It is expected that the high temperature inside the furnace should result the conversion of $CaCO_3$ present in the *Kapardika* powder into CaO [15] involving the reaction- (1) as follows.

   $CaCO_3 \rightarrow CaO + CO_2$ ---------- (1)

   But the *Kapardika bhasma* mainly shows the presence of $CaCO_3$ in the form of calcite along with $Ca(OH)_2$ in some *bhasma* samples. This observation is consistent with the observations reported by Ketakar etal and Dubey *etal* who have done rigorous investigation of *Mukta Shouktik bhasma*. [13,14] which is also a calcium carbonate based Ayurvedic *bhasma*. The repeated treatment of Aloe vera gel containing enough amount of water may prevent the decarboation of intermediate product of *Kapardika bhasma* [16]. Possibility of decarbonation of *Kapardika* powder in the form of aragonite and reformation of calcium carbonate in form of calcite via calcium hydroxide is also reported by Mishra etal and Krishna etal.[5] Calcium oxide formed due to calcinations in Reaction (1) has a great affinity for water to give $Ca(OH)_2$ through the reaction (2)

   $CaO + H_2O \rightarrow Ca(OH)_2$ -------- (2)

   $Ca(OH)_2 + CO_2 \rightarrow CaCO_3$ ------- (3)

   This $Ca(OH)_2$ again reacts with CO_2 present in the sealed earthen pot to give again $CaCO_3$ by reaction(3). The reactions (1), (2) and (3) take place simultaneously. But since the reaction is taking place in closed

system reaction (3) could be dominant due to presence of accumulated CO_2 in the earthen pot. This observation is also consistent with the detailed studies done by wadekar *etal* [2] on effect of calcinations cycles on preparation of vanga *bhasma* where the *bhasma* contains $CaCO_3$ along with tin oxide.

5. The peaks around 2870-2890 $cm^{-1}$are assignable to the asymmetric and symmetric stretching vibrations of sp^3 hybridized C-H bands and the peaks around 2509-2513 cm^{-1} are attributed to organic matters which contained hydroxyl matter. [10]

3.3 Electronic Spectroscopy

Solid state UV-Visible spectra of *Kapardika* powder and samples of *Kapardika bhasmas* are compared with standard $CaCO_3$ and Spectra of all the samples are presented in Fig-3 and important wavelengths along with their tentative assignments are shown in Table-3

.

Table-3 Solid State Electronic Spectra (λ max in nm) of *Kapardika* powder and *Kapardika bhasmas*.

Compound	Peak-1	Peak-2	Peak-3	Peak-4	Peak-5
Assignments	**σ-π***	**σ-π***	**π-π***	**π-π***	**n-π***
CaCO3	---	239	270	302 , 344	---
K	---	---	266	340	402
K-1	222	239	270	344	418
K-2	222	239	270	344	399
K-3	---	239	270	344	538
K-4	224	239	270	344	407

3.4 X- ray powder Diffraction

The XRD patterns of $CaCO_3$, *Kapardika powder* and four samples of *Kapardika bhasma* , are illustrated in Fig- 4. These XRD patterns show that all the samples include $CaCO_3$ as their major component. These patterns are composed of sharp lines with different intensities. From the nature of these lines it reveals that all these samples are crystalline in nature. The diffraction peak at (2theta=30.34^{0}A) *Kapardika* powder and the diffraction peaks (2 theta=29.78-28.99^{0}A) in all samples of *bhasma* indicate possibility of calcite phase of calcium carbonate [10,14]. The Particle size calculated from these XRD patterns using Scherrer Equation are shown in Table-4 which falls in the range 28 nm to 40 nm indicating their nanometric nature. This size is lower than the standard $CaCO_3$.

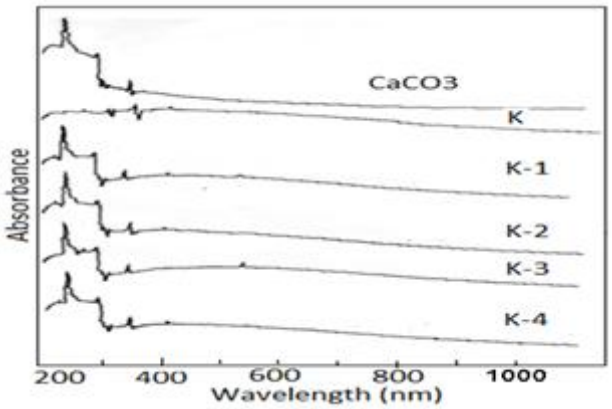


Fig -3 Electronic spectra of *Kapardika* powder and *Kapardika bhasma.*

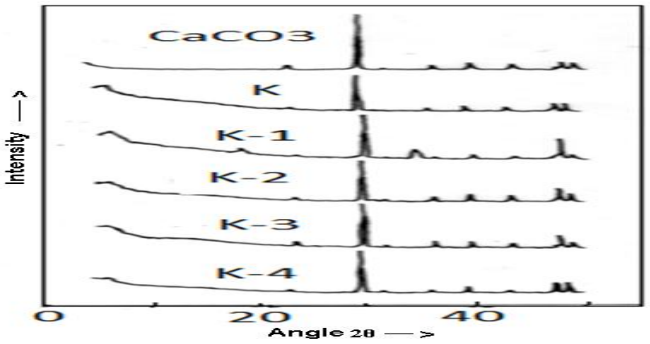


Fig-4 XRD patterns of *Kapardika* powder and *Kapardika bhasmas.*

Table- 4 Particle Size of *Kapardika* and *Kapardika bhasmas*

Sr. No	Name of the Compound	FWHM	Angel 2θ	Particle Size (nm)
1	$CaCO_3$	0.1897	29.5421	43.32
2	K	0.2544	30.3427	32.36
3	K-1	0.2096	29.7876	39.23
4	K-2	0.2929	29.5594	28.06
5	K-3	0.2307	29.7450	35.64
6	K-4	0.2582	28.9998	31.79

3.5 Thermo-gravimetric Analysis

Thermo-gravimetric analysis patterns of *Kapardika* powder and respective *bhasmas* are presented in Fig-5 and the important conclusions are summarized in Table-5 which supports $CaCO_3$ as major component in them along with small amount of organic matter and water or calcium hydroxide. Weight losses for CO_2 and weight of residues remaining at the end as CaO are matching well with the calculated weights. These results indicate that *Kapardika* powder contains 83% $CaCO_3$ and the *bhasma* samples contain 98-99% $CaCO_3$.

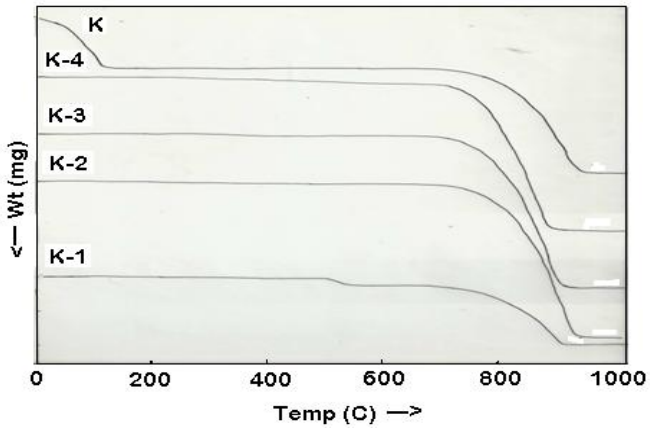


Fig-5 Thermo-gravimetric patterns of *Kapardika* powder and *Kapardika bhasmas*

3.6 SEM Studies

The SEM photographs of pure $CaCO_3$, *Kapardika* powder and samples of *Kapardika bhasma* at three magnifications are shown in Fig-6. A comparison of the SEM photographs of *Kapardika* powder with the SEM photographs of *Kapardika bhasma* shows that samples of *Kapardika bhasma* exhibits remarkable difference in their morphology. They seems to be composed of lumps or agglomeration which are heterogeneous in nature consisting of different sizes of crystallites .The conclusions from SEM studies may be summarized as follows: (i) The *Kapardika* powder is a mixture of square shaped and rod shaped particles and the particle size ranges in between 0.5 to 1micron. The reason of such mix particles could be, naturally occurring *Kapardika* is the hardest material among all the marine products and it is more difficult to transform it into finely divided powder form .

Due to this reason, special methods for the ayurvedic purification of *Kapardika* itself and its crude powder are recommended to transform it into finely divided powder form.

Table-5 Thermal decomposition Steps of *Kapardika* powder and *Kapardika bhasmas*.

Compound with initial wt	Decomposition Steps	Temperature range ^{0}C	Wt loss (mg)	Assignments
K 43mg	I	50-140	7.00	Loss due to organic matter and lattice water.
	II	700-925	15.10 (15.84)	Loss due to CO_2 from $CaCO_3$
				Residue as CaO = 21.00 (20.16)mg
K-1 10mg	I	50-460	0.10	Loss due to organic matter
	II	460-501	0.40	Loss due to organic matter
	III	650-850	3.50 (4.18)	Loss due to CO_2 from $CaCO_3$ and from small amount of $Ca(OH)_2$
				Residue as CaO=6.00 (5.32) mg
K-2 40mg	I	50-375	0.30	Loss due to organic matter
	II	800-925	17.20 (17.60)	Loss due to CO_2 from $CaCO_3$
				Residue as CaO=22.5 (22.4) mg
K-3 19.50 mg	I	50-375	0.1	Loss due to organic matter
	II	720-900	8.60 (8.53)	Loss due to CO_2 from $CaCO_3$
				Residue as CaO=12.80 (12.86) mg
K-4 13.00 mg	I	50-540	0.4	Loss due to organic matter
	II	725-875	5.60 (5.72)	Loss due to CO_2 from $CaCO_3$
				Residue as CaO=7.00 (7.20) mg

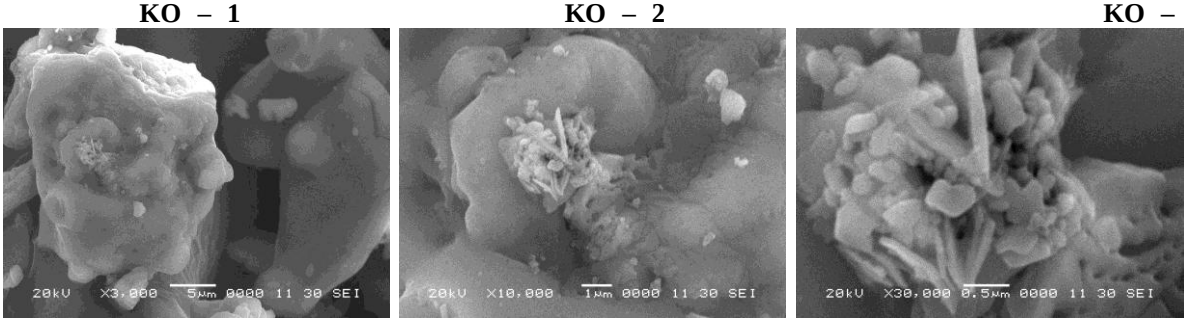
The values in the parentheses indicate calculated values.

(ii) The K-1 sample of *bhasma* shows high degree of agglomeration and the particle size ranges between 0.5 to 0.7 micron. These agglomerates appear to be square shaped and porous.
(iii) K-2 sample is composed of porous agglomerates and particle size is ranging from 0.7 to 0.1 micron. The shape of the particles is circular granules.
(iv) In K-3 also agglomeration is observed and the big particles are covered by small dusty particles as that of for K-2.The particle size ranges from 0.5 to 1micron.
(v) The K-4 sample shows particles of 0.5 to 0.7 micron with loss of their grain boundaries. Higher degree of agglomeration is a consequence of repeated calcinations cycles during the preparation of these *bhasmas* [2].

IV Conclusions

A comparative study of *Kapardika* powder and four commercial samples of *Kapardika bhasma* are carried out using modern instrumental techniques. These samples are mainly composed of calcium carbonate as indicated by EDX, IR and TGA. TGA is found to be the most useful technique for compositional studies of these *bhasmas*. Four samples of *Kapardika bhasma* resembles with each other with minor differences due to presence of small quantity of water, calcium hydroxide and organic matter. XRD of the four samples match with calcite phase of calcium carbonate and indicate nanometric nature of the *bhasmas.* Such nanometric Size could be responsible for the excellent therapeutic properties of the bhasmas.Further presence of organic matter as shown by IR and TG is likely to influence the bioassemibility of the bhasma. SEM analysis reveals agglomeration of the particles as consequences of repeated calcination cycles subjected during preparation of these *bhasmas.* Morphology of these samples is remarkably different from each other as seen from SEM photographs.

KO – 1 **KO – 2** **KO – 3**



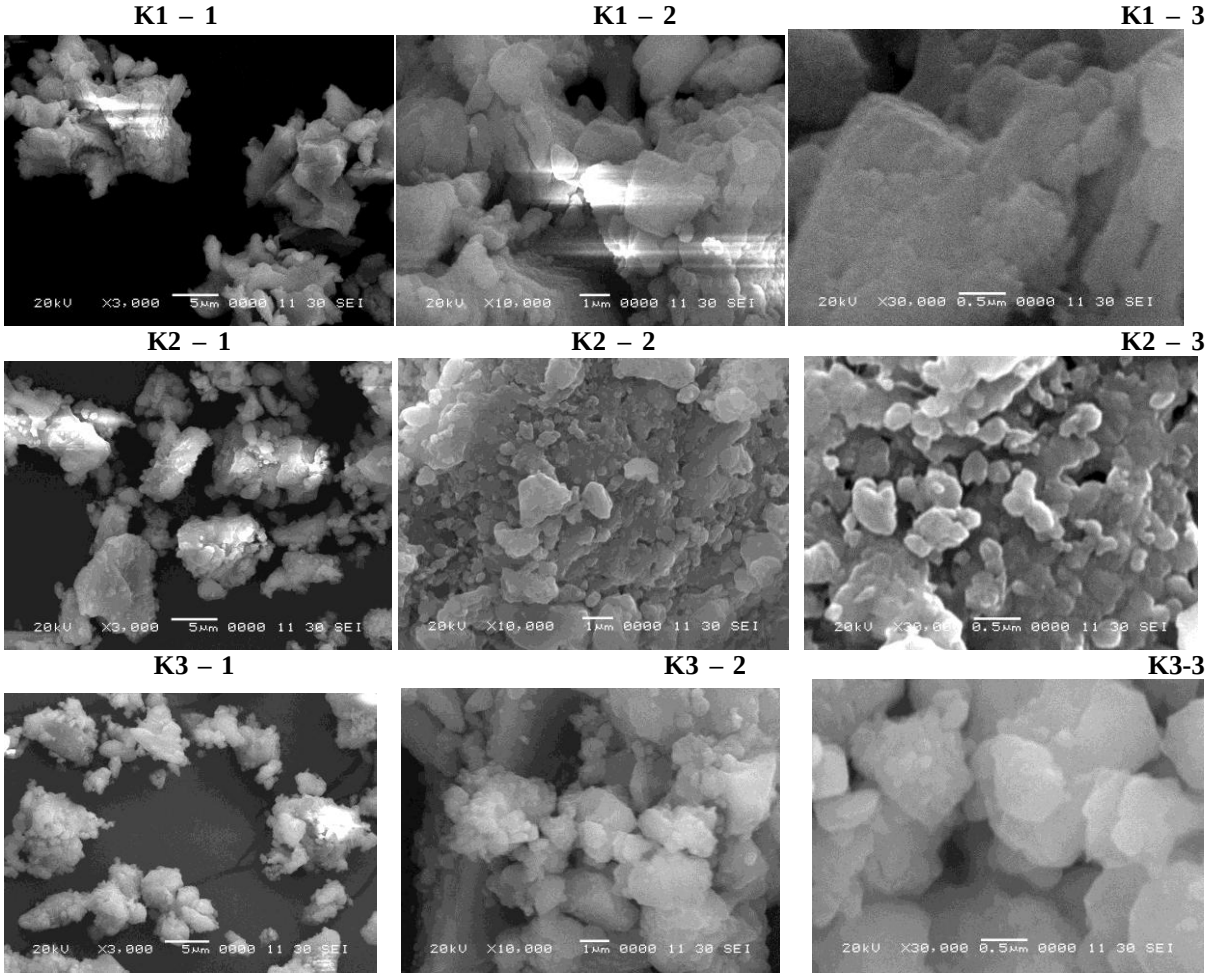


Fig-6 SEM photographs of *Kapardika* powder and *Kapardika bhasmas K-1,K-2, K-3*.

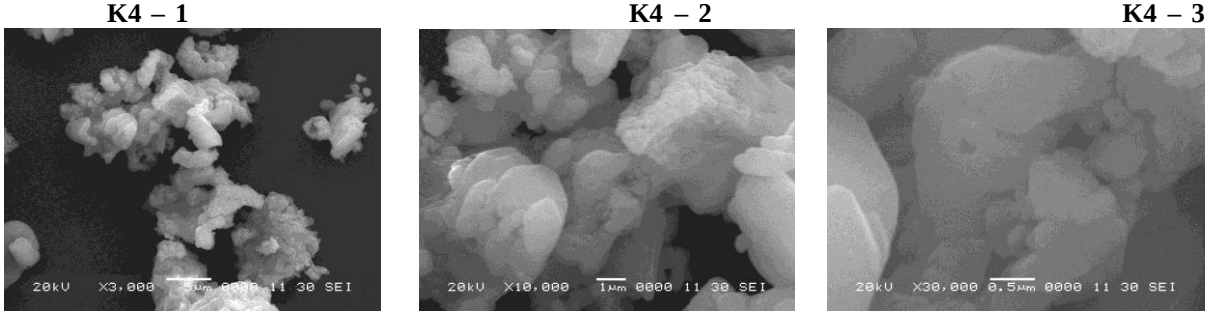


Fig-6 SEM photographs of *Kapardika bhasma sample K-4*

These findings may help the preparation of standard and reproducible formulation of *kapardika bhasma.*

Acknowledgements

Authors are thankful to Dr. S.S. Kadam, Vice Chancellor, Bharati Vidyapeeth Deemed University, Pune, Prof. K.D. Jadhav, Principal, Y.M. College, Pune, for providing necessary facilities and constant encouragement. Authors are also thankful to Dr. P.S. Khandagale and Dr. P.K. Khanna for their valuable suggestions.

References

[1] Wadekar M.P., Rode C.V., Bendale Y.N, Patil K.R., Prabhune A.A., Preparation and characterization of a copper based Indian traditional drug: Tamra *bhasma*. *Journal of pharmaceutical and biomedical analysis*, 39,2005,951-955.

[2] Wadekar M.P., Rode C.V., Bendale Y.N., Patil K.R., Gaikwad, Prabhune A.A., Effect of calcinations cycles on the preparation of tin oxide based traditional drug: Studies on its formation and characterization., *Journal of pharmaceutical and biomedical analysis*.,41,2006,1473-1478.

[3] Mrudula Wadekar, Vishwas Gogte, Prasad Khandagale and Asmita Prabhune. Comparative study of some commercial samples of Naga *Bhasma*., *Ancient Science of Life*, 23(4),2004, 45-48.

[4] Mrudula Kulkarni, G.T. Panse, B.A. Kulkarni and Yogesh Bendale. Characterization of Raupya *Bhasma* by using modern analytical techniques., *Journal of Indian Medicine and Homeopathy*, January-March, 2004,37-43.
[5] Kulshrestha Mayanka Krishna, Karbal Kamleshwar Singh., A critical review on Ayurvedic drug *Kapardika* (Cypraea Moneta Linn), *International research Journal of Pharmacy*, 3(10), 2012.
[6] Devanathan R., Rajalakshmi P. P. and Brindha P., Chemical Standardisation studies on Varatika *Bhasma*, *International Journal of Pharmaceutical research*, 2(4), 2010, 12-16.
[7] Kumar A., Nair A. G.C., Reddy A. V. R. and Garg A. N., Availability of essential elements in *bhasmas*: Analysis of Ayurvedic metallic preparations by INAA, *Journal of Radio-analytical and Nuclear Chemistry*, (270)1, 2006, 173-180.
[8] A. J. Baxi and S. A. Vasavada, Antacid activity of some Ayurvedic calcium preparations, *Indian J. Pharmacy*, **27,** 1965,227–230.
[9] Flemming A. Andersen and Ljerka Brecevic, Infrared spectra of amorphous and crystalline calcium carbonate, *Acta Chemica Scandinavica* ,45,1991, 1018-1024.
[10] Wenlong Wu, Liping Zhang, Zhijun Yang and Weisheng Hou., Study on microstructure of the Pinctada martensii pearls and its significance., *International Scholarly Research Network ISRN spectroscopy*, article ID 756217, 2012 ,9.
[11] FTIR and XRD analysis of mineral and organic matrix during heating of mother pearl from shell of the mollusc pinctada maxima., Balmain J., Hannoyer B and Lopez E, *Journal of Biomedical material Research*, 48(5), 1999,749-754.
[12] Meena Devi, P. Nagendra Prasad and K. Kalirajan., Infrared spectral studies on siddha drug-Pavalaparpam., *International journal of pharma and biosciences*, 1(4), 2010, 474-483.
[13] Ketkar A R, Kadam H.M. and Paradkar A.R., Temperature variability during preparation of shouktic *bhasma* and its consequences, *Indian Drugs*. 40(6),2003, 363-365.
[14] Nitin Dubey, Nidhi Dubey, Rajenndra S. Mehta, Ajay K. Saluja and Dinesh Jain., Physiological and pharmacological assessment of a traditional biomedicine: Mukta shouktic *bhasma*., *Songklanakarin J. Sci, Technol*,31(5), 2009,501-510.
[15] Engin, B. Demirtas H., Eken M., Temperature effects on egg shells investigated by XRD, IR and ESR techniques., *Radiation physics and chemistry*.,75,2006, 268-277.
[16] Ketkar A R, Borde P. P., Kadam H.M. and Paradkar A.R., Role of Aloe vera juice treatment(bhavana) in preparation of shouktic *bhasma*., *Indian Drugs*.,39(12), 2002, 661-663.